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NEWS 2		"Ask CAS" for self-help around the clock
NEWS 3	SEP 09	CA/CAplus records now contain indexing from 1907 to the present
NEWS 4	DEC 08	INPADOC: Legal Status data reloaded
NEWS 5	SEP 29	DISSABS now available on STN
NEWS 6	OCT 10	PCTFULL: Two new display fields added
NEWS 7	OCT 21	BIOSIS file reloaded and enhanced
NEWS 8	OCT 28	BIOSIS file segment of TOXCENTER reloaded and enhanced
NEWS 9	NOV 24	MSDS-CCOHS file reloaded
NEWS 10	DEC 08	CABA reloaded with left truncation
NEWS 11	DEC 08	IMS file names changed
NEWS 12	DEC 09	Experimental property data collected by CAS now available in REGISTRY
NEWS 13	DEC 09	STN Entry Date available for display in REGISTRY and CA/CAplus
NEWS 14	DEC 17	DGENE: Two new display fields added
NEWS 15	DEC 18	BIOTECHNO no longer updated
NEWS 16	DEC 19	CROPU no longer updated; subscriber discount no longer available
NEWS 17	DEC 22	Additional INPI reactions and pre-1907 documents added to CAS databases
NEWS 18	DEC 22	IFIPAT/IFIUDB/IFICDB reloaded with new data and search fields
NEWS 19	DEC 22	ABI-INFORM now available on STN
NEWS 20	JAN 27	Source of Registration (SR) information in REGISTRY updated and searchable
NEWS 21	JAN 27	A new search aid, the Company Name Thesaurus, available in CA/CAplus
NEWS 22	FEB 05	German (DE) application and patent publication number format changes

NEWS EXPRESS	DECEMBER 28 CURRENT WINDOWS VERSION IS V7.00, CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP), AND CURRENT DISCOVER FILE IS DATED 23 SEPTEMBER 2003
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

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  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 15:48:20 ON 19 FEB 2004

=> file medline, uspatful, dgene,embase, wpids, fsta, jicst, biosis, hcaplus
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FULL ESTIMATED COST                                       0.21       0.21

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=> s GFP
L1       43171 GFP

=> s GFP analogue
L2           5 GFP ANALOGUE

=> s Chromophore and l1
L3        1458 CHROMOPHORE AND L1

=> d l2 ti abs ibib tot

L2   ANSWER 1 OF 5  USPATFULL on STN
TI     Fluorescent proteins
AB     The present invention provides novel engineered derivatives of green
       fluorescent protein (GFP) which have an amino acid sequence which is
       modified by amino acid substitution compared with the amino acid
       sequence of wild type Green Fluorescent Protein. The modified GFPs
       exhibit enhanced fluorescence relative to wtGFP when expressed in
       non-homologous cells at temperatures above 30° C., and when
       excited at about 490 nm compared to the parent proteins, i.e. wtGFP. An
       example of a preferred protein is F64L-S175G-E222G-GFP. The modified
       GFPs provide a means for detecting GFP reporters in mammalian cells at
       lower levels of expression and/or increased sensitivity relative to
       wtGFP. This greatly improves the usefulness of fluorescent proteins in
       studying cellular functions in living cells.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
```

ACCESSION NUMBER: 2003:251073 USPATFULL
TITLE: Fluorescent proteins
INVENTOR(S): Stubbs, Simon Lawrence John, Amersham Buckinghamshire, UNITED KINGDOM
Jones, Anne Elizabeth, Amersham Buckinghamshire, UNITED KINGDOM
Michael, Nigel Paul, Amersham Buckinghamshire, UNITED KINGDOM
Thomas, Nicholas, Amersham Buckinghamshire, UNITED KINGDOM

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003175859	A1	20030918	
APPLICATION INFO.:	US 2001-967301	A1	20010928	(9)

	NUMBER	DATE
PRIORITY INFORMATION:	GB 2001-9858	20010423

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: AMERSHAM BIOSCIENCES, PATENT DEPARTMENT, 800 CENTENNIAL AVENUE, PISCATAWAY, NJ, 08855
NUMBER OF CLAIMS: 24
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Page(s)
LINE COUNT: 1284
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 2 OF 5 USPATFULL on STN
TI Novel fluorescent proteins
AB A GFP with an F64L mutation and an E222G mutation is provided. This GFP has a bigger Stokes shift compared to other GFPs making it very suitable for high throughput screening due to a better resolution. This GFP also has an excitation maximum between the yellow GFP and the cyan GFP allowing for cleaner band separation when used together with those GFPs.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:314712 USPATFULL
TITLE: Novel fluorescent proteins
INVENTOR(S): Bjorn, Sara Petersen, Lyngby, DENMARK
Pagliaro, Len, Copenhagen K, DENMARK
Thastrup, Ole, Birkerod, DENMARK

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002177189	A1	20021128	
APPLICATION INFO.:	US 2001-887784	A1	20010619	(9)

	NUMBER	DATE	
PRIORITY INFORMATION:	DK 2000-953	20000619	
	DK 2001-739	20010510	
	US 2000-212681P	20000620	(60)
	US 2001-290170P	20010510	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: BIRCH STEWART KOLASCH & BIRCH, PO BOX 747, FALLS CHURCH, VA, 22040-0747
NUMBER OF CLAIMS: 19
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 8 Drawing Page(s)
LINE COUNT: 1225
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 3 OF 5 USPATFULL on STN
TI Novel fluorescent proteins
AB The present invention relates to novel variants of the fluorescent protein GFP having improved fluorescence properties.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:199251 USPATFULL
TITLE:	Novel fluorescent proteins
INVENTOR(S):	Thastrup, Ole, Birkerod, DENMARK Tullin, Soren, Soborg, DENMARK Poulsen, Lars Kongsbak, Holte, DENMARK Bjorn, Sara Petersen, Lyngby, DENMARK
PATENT ASSIGNEE(S):	BIOIMAGE A/S (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002107362	A1	20020808
APPLICATION INFO.:	US 2001-872364	A1	20010601 (9)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 2000-619310, filed on 19 Jul 2000, PENDING Continuation of Ser. No. US 1997-819612, filed on 17 Mar 1997, GRANTED, Pat. No. US 6172188 Continuation of Ser. No. WO 1996-DK51, filed on 31 Jan 1996, UNKNOWN		

	NUMBER	DATE
PRIORITY INFORMATION:	DK 1995-1065	19950922

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	BIRCH STEWART KOLASCH & BIRCH, PO BOX 747, FALLS CHURCH, VA, 22040-0747
NUMBER OF CLAIMS:	25
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	12 Drawing Page(s)
LINE COUNT:	1239

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 4 OF 5 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI Nucleic acid reporter construct useful for transfecting host cell comprises a nucleic acid sequence encoding a detectable live-cell reporter molecule.
AN 2003-403105 [38] WPIDS
AB WO2003031612 A UPAB: 20030616
NOVELTY - A nucleic acid reporter construct (I) comprises a nucleic acid sequence encoding a detectable live-cell reporter molecule linked to and under the control of at least one cell cycle phase-specific expression control element, and a destruction control element. The reporter construct is expressed in a cell at a predetermined position in the cell cycle.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) a nucleic acid reporter construct (II) comprising an expression vector which comprises:

(a) a vector backbone comprising a bacterial origin of replication and a bacterial drug resistance gene;

(b) a cell cycle phase specific expression control element;

(c) a destruction control element; and

(d) a nucleic acid sequence encoding a reporter molecule;

(2) a host cell transfected with (I) or (II);

(3) determining the cell cycle position of a cell involving expressing (I), and determining the cell cycle position by monitoring signals emitted by the reporter molecule;

(4) determining the effect of a test system on the cell cycle position of a cell involving:

(a) expressing (I) in the cell in the absence or the presence of the test system, and determining the cell cycle position by monitoring signals emitted by the reporter molecule, where a difference between the emitted signals measured in the absence or in the presence of the test system is indicative of the effect of the test system on the cell cycle position of the cell; or

(b) expressing (I) in the cell in the absence or the presence of the test system, determining the cell cycle position by monitoring signals emitted by the reporter molecule, and comparing the emitted signal in the presence of the test system with a known value for the emitted signal in the absence of the test system, where a difference between the emitted signal measured in the presence of the test system and the known value in the absence of the test system is indicative of the effect of the test system on the cell cycle position of the cell; or

(c) providing cells containing (I), culturing first and second population of the cells in the presence and absence, respectively, of a test system and under conditions permitting expression of (I), and measuring the signals emitted by the reporter molecule in both populations, where a difference between the emitted signals in between both populations is indicative of the test system on the cell cycle position of the cell;

(5) a method of determining the effect of the cell cycle on the expression, translocation, or sub-cellular distribution of a first detectable reporter which is known to vary in response to a test system, comprising:

(a) expressing in the cell in the presence of the test system a second nucleic acid reporter construct;

(b) determining the cell cycle position by monitoring signals emitted by the second reporter molecule; and

(c) monitoring the signals emitted by the first detectable reporter, where the relationship between cell cycle position determined by step (b) and the signal emitted by the first detectable reporter indicates the expression, translocation or sub-cellular distribution;

(6) a cell line comprising the host cell useful in establishing xenografts or allografts in a host organism; and

(7) a transgenic organism comprising the host cell.

ACTIVITY - Immunosuppressive.

MECHANISM OF ACTION - Transfected host cell cycle blocker.

U2OS cells (ATCC HTB-96) were cultured in 96 well microtitre plate. Cells were transfected with a cell cycle reporter construct comprising a cyclin B1 promoter linked to sequence encoding the cyclin B1 D-box, cyclin B1 CRS and green fluorescent protein (GFP) in a pCORON4004 vector using Fugene 6 as the transfection agent. After 24 hours of culture, the demecolcine (test) cell was exposed to culture media and control cell was exposed to culture media alone. Cells were incubated for 24 hours and then analyzed for nuclear GFP expression using a confocal scanning imager with automated image analysis. The percent positive (test)/(control) cells were approximately 15/10.

USE - For transfecting host cell (e.g. eukaryotic cell, mammalian cell, fungal cell, nematode cell, fish cell, amphibian cell, plant cell and an insect cell) which is useful in establishing xenografts or allografts in a host organism (claimed).

ADVANTAGE - The construct allows improved, non-destructive and dynamic determination of cell cycle phase status and allows continuous monitoring of cell cycle progression in individual cells.

Dwg.0/10

ACCESSION NUMBER: 2003-403105 [38] WPIDS
DOC. NO. CPI: C2003-107325
TITLE: Nucleic acid reporter construct useful for transfecting host cell comprises a nucleic acid sequence encoding a detectable live-cell reporter molecule.
DERWENT CLASS: B04 D16
INVENTOR(S): FRANCIS, M J; GOODYER, I D; ISMAIL, R A; JONES, A E; KENDALL, J M; PINES, J N; THOMAS, N

PATENT ASSIGNEE(S): (AMSH) AMERSHAM BIOSCIENCES UK LTD; (CANC-N) CANCER RES TECHNOLOGY LTD
COUNTRY COUNT: 101
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2003031612	A2	20030417	(200338)*	EN	26

RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003031612	A2	WO 2002-GB4258	20020912

PRIORITY APPLN. INFO: GB 2001-23856 20011005

L2 ANSWER 5 OF 5 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI Measurements of (Ca2+)i in cells and tissues of transgenic mice expressing the calcium sensitive **GFP analogue** G-CaMP restricted to smooth muscle.
ACCESSION NUMBER: 2003:409881 BIOSIS
DOCUMENT NUMBER: PREV200300409881
TITLE: Measurements of (Ca2+)i in cells and tissues of transgenic mice expressing the calcium sensitive **GFP analogue** G-CaMP restricted to smooth muscle.
AUTHOR(S): Kotlikoff, M. I. [Reprint Author]; Nakai, J.; Su, K. [Reprint Author]; Deng, K.-Y. [Reprint Author]; Lee, J. C. [Reprint Author]; Wilson, J. [Reprint Author]; Feldman, M. E. [Reprint Author]; Ji, G.-J. [Reprint Author]
CORPORATE SOURCE: Cornell Univ, T4018 VRT, Box 11, Ithaca, NY, 14853, USA
SOURCE: Biophysical Journal, (February 2003) Vol. 84, No. 2 Part 2, pp. 144a. print.
Meeting Info.: 47th Annual Meeting of the Biophysical Society. San Antonio, TX, USA. March 01-05, 2003. Biophysical Society.
ISSN: 0006-3495 (ISSN print).
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Sep 2003
Last Updated on STN: 10 Sep 2003

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FILE 'MEDLINE, USPATFULL, DGENE, EMBASE, WPIDS, FSTA, JICST-EPLUS, BIOSIS, HCAPLUS' ENTERED AT 15:48:47 ON 19 FEB 2004
L1 43171 S GFP
L2 5 S GFP ANALOGUE
L3 1458 S CHROMOPHORE AND L1

=> s l3 and substitution
L4 369 L3 AND SUBSTITUTION

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=> s l4 and position 64
L5          13 L4 AND POSITION 64

=> d l5 ti abs ibib tot

L5   ANSWER 1 OF 13  USPATFULL on STN
TI     Bioluminescence resonance energy transfer( bret) system with broad
       spectral resolution between donor and acceptor emission wavelengths and
       its use
AB     The present invention provides a bioluminescence resonance energy
       transfer (BRET) detection system characterised by a broad spectral
       resolution between donor and acceptor emission wavelengths. The broad
       spectral resolution between the emission wavelength of the
       bioluminescent donor protein and the fluorescent acceptor molecule
       results in an increased signal-to-base ratio and dynamic range in
       comparison with a basic BRET system.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER:        2003:288646  USPATFULL
TITLE:                   Bioluminescence resonance energy transfer( bret) system
                         with broad spectral resolution between donor and
                         acceptor emission wavelengths and its use
INVENTOR(S):             Joly, Erik, Blainville, CANADA

                              NUMBER        KIND    DATE
                         ---------------- ------ --------
PATENT INFORMATION:      US 2003203404      A1   20031030
APPLICATION INFO.:       US 2002-168447     A1   20021218  (10)
                         WO 2000-CA1516          20001222

                               NUMBER         DATE
                         ------------------ --------
PRIORITY INFORMATION:    CA 1999-2291968    19991222
                         CA 2000-2314861    20000802
DOCUMENT TYPE:           Utility
FILE SEGMENT:            APPLICATION
LEGAL REPRESENTATIVE:    Hollie L Baker, Hale and Dorr, 60 State Street, Boston,
                         MA, 02109
NUMBER OF CLAIMS:        34
EXEMPLARY CLAIM:         1
NUMBER OF DRAWINGS:      34 Drawing Page(s)
LINE COUNT:              3234
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5   ANSWER 2 OF 13  USPATFULL on STN
TI     Mutants of Green Fluorescent Protein
AB     The present invention provides mutants of the Green Fluorescent Protein
       (GFP) of Aequorea victoria. Specifically provided by the
       present invention are nucleic acid molecules encoding mutant GFPs, the
       mutant GFPs encoded by these nucleic acid molecules, vectors and host
       cells comprising these nucleic acid molecules, and kits comprising one
       or more of the above as components. The invention also provides methods
       for producing these mutant GFPs. The fluorescence of these mutants is
       observable using fluorescein optics, making the mutant proteins of the
       present invention available for use in techniques such as fluorescence
       microscopy and flow cytometry using standard FITC filter sets. In
       addition, certain of these mutant proteins fluoresce when illuminated by
       white light, particularly when expressed at high levels in prokaryotic
       or eukaryotic host cells or when present in solution or in purified form
       at high concentrations. The mutant GFP sequences and peptides
       of the present invention are useful in the detection of transfection, in
       fluorescent labeling of proteins, in construction of fusion proteins
       allowing examination of intracellular protein expression, biochemistry
```

and trafficking, and in other applications requiring the use of reporter genes.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:285169 USPATFULL
TITLE: Mutants of Green Fluorescent Protein
INVENTOR(S): Evans, Krista, Germantown, MD, United States
PATENT ASSIGNEE(S): Invitrogen Corporation, Carlsbad, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6638732	B1	20031028
APPLICATION INFO.:	US 1999-472065		19991223 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1997-970762, filed on 14 Nov 1997, now abandoned

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-30935P	19961115 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Slobodyansky, Elizabeth
LEGAL REPRESENTATIVE: Sterne, Kessler, Goldstein & Fox P.L.L.C.
NUMBER OF CLAIMS: 23
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 12 Drawing Figure(s); 12 Drawing Page(s)
LINE COUNT: 1614
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 3 OF 13 USPATFULL on STN
TI Fluorescent proteins
AB The present invention provides novel engineered derivatives of green fluorescent protein (**GFP**) which have an amino acid sequence which is modified by amino acid **substitution** compared with the amino acid sequence of wild type Green Fluorescent Protein. The modified GFPs exhibit enhanced fluorescence relative to wtGFP when expressed in non-homologous cells at temperatures above 30° C., and when excited at about 490 nm compared to the parent proteins, i.e. wtGFP. An example of a preferred protein is F64L-S175G-E222G-**GFP**. The modified GFPs provide a means for detecting **GFP** reporters in mammalian cells at lower levels of expression and/or increased sensitivity relative to wtGFP. This greatly improves the usefulness of fluorescent proteins in studying cellular functions in living cells.

bad date

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:251073 USPATFULL
TITLE: Fluorescent proteins
INVENTOR(S): Stubbs, Simon Lawrence John, Amersham Buckinghamshire, UNITED KINGDOM
Jones, Anne Elizabeth, Amersham Buckinghamshire, UNITED KINGDOM
Michael, Nigel Paul, Amersham Buckinghamshire, UNITED KINGDOM
Thomas, Nicholas, Amersham Buckinghamshire, UNITED KINGDOM

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003175859	A1	20030918
APPLICATION INFO.:	US 2001-967301	A1	20010928 (9)

	NUMBER	DATE

PRIORITY INFORMATION: GB 2001-9858 20010423
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: AMERSHAM BIOSCIENCES, PATENT DEPARTMENT, 800 CENTENNIAL AVENUE, PISCATAWAY, NJ, 08855
NUMBER OF CLAIMS: 24
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Page(s)
LINE COUNT: 1284
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 4 OF 13 USPATFULL on STN
TI Non-oligomerizing fluorescent proteins
AB A non-oligomerizing fluorescent protein, which is derived from a fluorescent protein having at least one mutation that reduces or eliminates the ability of the fluorescent protein to oligomerize, is provided. The non-oligomerizing fluorescent protein can be derived from a naturally occurring green fluorescent protein, a red fluorescent protein, or other fluorescent protein, or a fluorescent protein related thereto. Also provided is a fusion protein, which includes a non-oligomerizing fluorescent protein linked to at least one polypeptide of interest. In addition, a polynucleotide encoding a non-oligomerizing fluorescent protein is provided, as is a recombinant nucleic acid molecule, which includes polynucleotide encoding a non-oligomerizing fluorescent protein operatively linked to at least a second polynucleotide. Vectors and host cells containing such polynucleotides also are provided, as are kits containing one or more non-oligomerizing fluorescent proteins or encoding polynucleotides or constructs derived therefrom. Further provided are methods of making and using the proteins and polynucleotides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:244502 USPATFULL
TITLE: Non-oligomerizing fluorescent proteins
INVENTOR(S): Tsien, Roger Y., La Jolla, CA, UNITED STATES
Zacharias, David A., San Diego, CA, UNITED STATES
Baird, Geoffrey S., Solana Beach, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003170911	A1	20030911	
APPLICATION INFO.:	US 2001-794308	A1	20010226	(9)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614
NUMBER OF CLAIMS: 72
EXEMPLARY CLAIM: 1
LINE COUNT: 3003
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 5 OF 13 USPATFULL on STN
TI Non-oligomerizing tandem fluorescent proteins
AB Non-oligomerizing fluorescent proteins, which are formed by operatively linking two or more monomers of a fluorescent protein, or which are derived from a fluorescent protein having at least one mutation that reduces or eliminates the ability of the fluorescent protein to oligomerize, are provided. The non-oligomerizing fluorescent proteins can be derived from a naturally occurring green fluorescent protein, a red fluorescent protein, or other fluorescent protein, or a fluorescent protein related thereto. Also provided is a fusion protein, which includes a non-oligomerizing fluorescent protein linked to at least one polypeptide of interest. In addition, a polynucleotide encoding a non-oligomerizing fluorescent protein is provided, as is a recombinant

nucleic acid molecule, which includes polynucleotide encoding a non-oligomerizing fluorescent protein operatively linked to at least a second polynucleotide. Vectors and host cells containing such polynucleotides also are provided, as are kits containing one or more non-oligomerizing fluorescent proteins or encoding polynucleotides or constructs derived therefrom. Further provided are methods of making and using the proteins and polynucleotides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:44778 USPATFULL
TITLE: Non-oligomerizing tandem fluorescent proteins
INVENTOR(S): Tsien, Roger Y., La Jolla, CA, UNITED STATES
Campbell, Robert E., San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003032088	A1	20030213
APPLICATION INFO.:	US 2001-866538	A1	20010524 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2001-794308, filed on 26 Feb 2001, PENDING
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 620 NEWPORT CENTER DRIVE, SIXTEENTH FLOOR, NEWPORT BEACH, CA, 92660
NUMBER OF CLAIMS: 87
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 1 Drawing Page(s)
LINE COUNT: 3627
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 6 OF 13 USPATFULL on STN
TI Novel fluorescent proteins
AB A **GFP** with an F64L mutation and an E222G mutation is provided. This **GFP** has a bigger Stokes shift compared to other GFPs making it very suitable for high throughput screening due to a better resolution. This **GFP** also has an excitation maximum between the yellow **GFP** and the cyan **GFP** allowing for cleaner band separation when used together with those GFPs.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:314712 USPATFULL
TITLE: Novel fluorescent proteins
INVENTOR(S): Bjorn, Sara Petersen, Lyngby, DENMARK
Pagliaro, Len, Copenhagen K, DENMARK
Thastrup, Ole, Birkerod, DENMARK

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002177189	A1	20021128
APPLICATION INFO.:	US 2001-887784	A1	20010619 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	DK 2000-953	20000619
	DK 2001-739	20010510
	US 2000-212681P	20000620 (60)
	US 2001-290170P	20010510 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: BIRCH STEWART KOLASCH & BIRCH, PO BOX 747, FALLS CHURCH, VA, 22040-0747
NUMBER OF CLAIMS: 19
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 8 Drawing Page(s)

LINE COUNT: 1225
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 7 OF 13 USPATFULL on STN
TI Novel fluorescent proteins
AB The present invention relates to novel variants of the fluorescent protein **GFP** having improved fluorescence properties.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:199251 USPATFULL
TITLE: Novel fluorescent proteins
INVENTOR(S): Thastrup, Ole, Birkerod, DENMARK
Tullin, Soren, Soborg, DENMARK
Poulsen, Lars Kongsbak, Holte, DENMARK
Bjorn, Sara Petersen, Lyngby, DENMARK
PATENT ASSIGNEE(S): BIOIMAGE A/S (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002107362	A1	20020808
APPLICATION INFO.:	US 2001-872364	A1	20010601 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2000-619310, filed on 19 Jul 2000, PENDING Continuation of Ser. No. US 1997-819612, filed on 17 Mar 1997, GRANTED, Pat. No. US 6172188 Continuation of Ser. No. WO 1996-DK51, filed on 31 Jan 1996, UNKNOWN

	NUMBER	DATE
PRIORITY INFORMATION:	DK 1995-1065	19950922

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: BIRCH STEWART KOLASCH & BIRCH, PO BOX 747, FALLS CHURCH, VA, 22040-0747
NUMBER OF CLAIMS: 25
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 12 Drawing Page(s)
LINE COUNT: 1239
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 8 OF 13 USPATFULL on STN
TI Rapidly greening, low oxygen mutant of the aequoria victoria green fluorescent protein
AB A novel catagory of Green Fluorescent Protein (**GFP**) is disclosed, which exhibits the novel trait of rapid **chromophore** formation under reduced oxygen levels while maintaining a fluorescence spectrum similar to the wildtype **GFP**. Examples of this category of low oxygen variants are the mutations F64C, F64M and V68C. These **GFP** variants also exhibit the useful traits of thermotolerance and higher fluorescence yield. Mutants that combine this low oxygen **chromophore** development with mutations conferring blue or red fluorescence are also disclosed. These fluorescent proteins may be used in any of the applications where the wild type green fluorescent protein is used, as well as many new anaerobic applications where **GFP** could previously not be used.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:160848 USPATFULL
TITLE: Rapidly greening, low oxygen mutant of the aequoria victoria green fluorescent protein
INVENTOR(S): Fisher, Hugh, North Brunswick, NJ, United States
PATENT ASSIGNEE(S): Rutgers, the State University, New Brunswick, NJ, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6414119	B1	20020702
APPLICATION INFO.:	US 1999-418785		19991015 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-104563P	19981016 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Slobodyansky, Elizabeth
LEGAL REPRESENTATIVE: Licata & Tyrrell P.C.
NUMBER OF CLAIMS: 8
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 9 Drawing Figure(s); 7 Drawing Page(s)
LINE COUNT: 911
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 9 OF 13 USPATFULL on STN
TI Fluorescent proteins
AB The present invention relates to novel variants of the fluorescent protein **GFP** having improved fluorescence properties.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2001:4862 USPATFULL
TITLE: Fluorescent proteins
INVENTOR(S): Thastrup, Ole, Birkevej 37, DK-3460 Biker.o slashed.d, Denmark
Tullin, S.o slashed.ren, Solnavej 53, 1. tv., DK-2860-S.o slashed.borg, Denmark
Poulsen, Lars Kongsbak, V.ae butted.ngestien 2A, DK-2840 Holte, Denmark
Bj.o slashed.rn, Sara Petersen, Klampenborgvej 102, DK-2800 Lyngby, Denmark

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6172188	B1	20010109
APPLICATION INFO.:	US 1997-819612		19970317 (8)
RELATED APPLN. INFO.:	Continuation of Ser. No. WO 1996-DK51, filed on 31 Jan 1996		

	NUMBER	DATE
PRIORITY INFORMATION:	DK 1995-1065	19950922

DOCUMENT TYPE: Patent
FILE SEGMENT: Granted
PRIMARY EXAMINER: Ungar, Susan
NUMBER OF CLAIMS: 15
EXEMPLARY CLAIM: 1,7,8
NUMBER OF DRAWINGS: 12 Drawing Figure(s); 12 Drawing Page(s)
LINE COUNT: 831
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 10 OF 13 USPATFULL on STN
TI Mutants of the green fluorescent protein having improved fluorescent properties at 37°
AB The present invention relates to mutants of the green fluorescent protein having improved fluorescent properties at 37° C. The mutants provide for improved methods of monitoring gene expression, e.g., for use as cell markers or protein expression indicators in prokaryotic and, especially, eucaryotic systems where the standard physiological temperature is 37° C.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2000:98547 USPATFULL
TITLE: Mutants of the green fluorescent protein having improved fluorescent properties at 37°
INVENTOR(S): Michaels, Mark, Encino, CA, United States
PATENT ASSIGNEE(S): Amgen Inc., Thousand Oaks, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6096865		20000801
APPLICATION INFO.:	US 1996-643704		19960506 (8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Spector, Lorraine
ASSISTANT EXAMINER: Kaufman, Claire M.
LEGAL REPRESENTATIVE: Crandall, Craig A., Levy, Ron K., Odre, Steven M.
NUMBER OF CLAIMS: 6
EXEMPLARY CLAIM: 1
LINE COUNT: 1271
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 11 OF 13 USPATFULL on STN
TI FACS-optimized mutants of the green fluorescent protein (**GFP**)
AB Three classes of **GFP** mutants having single excitation maxima around 488 nm are brighter than wild-type **GFP** following 488 nm excitation. GFPmut1 has a double **substitution**: F64L, S65T; GFPmut2 has a triple **substitution**: S65A, V68L, S72A; and GFPmut3 is characterized by the double **substitution** S65G, S72A. The excitation maxima of the three mutants are at 488 nm, 481 nm and 501 nm respectively. The fluorescence intensities following excitation at 488 nm are an order of magnitude higher than that of wild-type **GFP** excited at 488 nm in E. coli. The expression of **GFP** is observable minutes after induction.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2000:92074 USPATFULL
TITLE: FACS-optimized mutants of the green fluorescent protein (**GFP**)
INVENTOR(S): Cormack, Brendan P., Santa Cruz, CA, United States
Valdivia, Raphael H., Palo Alto, CA, United States
Falkow, Stanley, Portola Valley, CA, United States
PATENT ASSIGNEE(S): The Board of Trustees of the Leland Stanford Junior University, Palo Alto, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6090919		20000718
APPLICATION INFO.:	US 1998-135418		19980817 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1997-791332, filed on 31 Jan 1997, now patented, Pat. No. US 5804387

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-10960P	19960201 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Prouty, Rebecca E.
ASSISTANT EXAMINER: Stole, Einar
LEGAL REPRESENTATIVE: Bozicevic, Field & Francis, Sherwood, Pamela
NUMBER OF CLAIMS: 15
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 5 Drawing Figure(s); 5 Drawing Page(s)

LINE COUNT: 862
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 12 OF 13 USPATFULL on STN
TI Humanized green fluorescent protein genes and methods
AB Disclosed are synthetic and "humanized" versions of green fluorescent protein (**GFP**) genes adapted for high level expression in mammalian cells, especially those of human origin. Base substitutions are made in various codons in order to change the codon usage to one more appropriate for expression in mammalian cells. Recombinant vectors carrying such humanized genes are also disclosed. In addition, various methods for using the efficient expression of humanized **GFP** in mammalian cells and in animals are described.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2000:12655 USPATFULL
TITLE: Humanized green fluorescent protein genes and methods
INVENTOR(S): Muzyczka, Nicholas, Gainesville, FL, United States
Zolotukhin, Sergei, Gainesville, FL, United States
Hauswirth, William, Gainesville, FL, United States
PATENT ASSIGNEE(S): University of Florida, Gainesville, FL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6020192		20000201
APPLICATION INFO.:	US 1997-893327		19970716 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1997-588201, filed on 18 Jan 1997
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Degen, Nancy
ASSISTANT EXAMINER: Wang, Andrew
LEGAL REPRESENTATIVE: Arnold, White & Durkee
NUMBER OF CLAIMS: 11
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 29 Drawing Figure(s); 22 Drawing Page(s)
LINE COUNT: 4342
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 13 OF 13 USPATFULL on STN
TI FACS-optimized mutants of the green fluorescent protein (**GFP**)
AB Three classes of **GFP** mutants having single excitation maxima around 488 nm are brighter than lid-type **GFP** following 488 nm excitation. GFPmut1 has a double **substitution**: F64L, S65T; GFPmut2 has a triple **substitution**: S65A, V68L, S72A; and GFPmut3 is characterized by the double **substitution** S65G, S72A. The excitation maxima of the three mutants are at 488 nm, 481 nm and 501 nm respectively. The fluorescence intensities following excitation at 488 nm are an order of magnitude higher than that of wild-type **GFP** excited at 488 nm in E. coli. The expression of **GFP** is observable minutes after induction.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 1998:108223 USPATFULL
TITLE: FACS-optimized mutants of the green fluorescent protein (**GFP**)
INVENTOR(S): Cormack, Brendan P., Santa Cruz, CA, United States
Valdivia, Raphael H., Palo Alto, CA, United States
Falkow, Stanley, Porola Valley, CA, United States
PATENT ASSIGNEE(S): The Board of Trustees of the Leland Stanford Junior University, Palo Alto, CA, United States (U.S. corporation)

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                          NUMBER         KIND    DATE
                          -------------- ------ --------
PATENT INFORMATION:       US 5804387              19980908
APPLICATION INFO.:        US 1997-791332          19970131  (8)

                          NUMBER         DATE
                          -------------- --------
PRIORITY INFORMATION:     US 1996-10960P 19960201 (60)
DOCUMENT TYPE:            Utility
FILE SEGMENT:             Granted
PRIMARY EXAMINER:         Hendricks, Keith D.
ASSISTANT EXAMINER:       Stole, Einar
LEGAL REPRESENTATIVE:     Bozicevic & Reed LLP
NUMBER OF CLAIMS:         50
EXEMPLARY CLAIM:          1
NUMBER OF DRAWINGS:       6 Drawing Figure(s); 5 Drawing Page(s)
LINE COUNT:               962
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


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     (FILE 'HOME' ENTERED AT 15:48:20 ON 19 FEB 2004)

     FILE 'MEDLINE, USPATFULL, DGENE, EMBASE, WPIDS, FSTA, JICST-EPLUS,
     BIOSIS, HCAPLUS' ENTERED AT 15:48:47 ON 19 FEB 2004
L1         43171 S GFP
L2             5 S GFP ANALOGUE
L3          1458 S CHROMOPHORE AND L1
L4           369 S L3 AND SUBSTITUTION
L5            13 S L4 AND POSITION 64

=> s l4 and E222G
L6            15 L4 AND E222G

=> s l4 and F64L
L7            51 L4 AND F64L

=> s l7 and l6
L8            11 L7 AND L6

=> d l8 ti abs ibib tot

L8   ANSWER 1 OF 11  USPATFULL on STN
TI     Long wavelength engineered fluorescent proteins
AB     Engineered fluorescent proteins, nucleic acids encoding them and methods
       of use.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER:         2004:18826  USPATFULL
TITLE:                    Long wavelength engineered fluorescent proteins
INVENTOR(S):              Wachter, Rebekka M., Creswell, OR, UNITED STATES
                          Remington, S. James, Eugene, OR, UNITED STATES

                          NUMBER         KIND    DATE
                          -------------- ------ --------
PATENT INFORMATION:       US 2004014128      A1  20040122
APPLICATION INFO.:        US 2003-620099     A1  20030714  (10)
RELATED APPLN. INFO.:     Division of Ser. No. US 2000-575847, filed on 19 May
                          2000, GRANTED, Pat. No. US 6593135 Continuation-in-part
                          of Ser. No. US 1997-974737, filed on 19 Nov 1997,
                          GRANTED, Pat. No. US 6077707 Continuation of Ser. No.
                          US 1997-911825, filed on 15 Aug 1997, GRANTED, Pat. No.
                          US 6054321 Continuation-in-part of Ser. No. US
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1996-706408, filed on 30 Aug 1996, GRANTED, Pat. No. US 6124128

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Lisa A. Haile, J.D., Ph.D., GRAY CARY WARE & FREIDENRICH LLP, Suite 1100, 4365 Executive Drive, San Diego, CA, 92121-2133
NUMBER OF CLAIMS: 187
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 62 Drawing Page(s)
LINE COUNT: 3919
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 2 OF 11 USPATFULL on STN
TI Live cell procedures to identify to identify compounds modulating intracellular distribution of phosphodiesterase(pde) enzymes
AB An alternative therapeutic approach for PDE4 inhibition is disclosed. PDE4 dislocators, will remove the PDE4 away from the native location in the cell and thereby increase the concentration of cAMP in this location. By dislocating the PDE4, and thereby not acting directly on the catalytic, among phosphodiesterase inhibitors, well conserved site, the compound will act e.g. at the binding domain of the PDE4, thereby providing isoform-specific `inhibitors` of PDE4. The dislocation of PDE4s are visualised with fusions to **GFP**. The native location is induced by treatment with Rolipram.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:266039 USPATFULL
TITLE: Live cell procedures to identify to identify compounds modulating intracellular distribution of phosphodiesterase(pde) enzymes
INVENTOR(S): Terry, Bernard Robert, Frederiksberg, DENMARK
Scudder, Kurt Marshall, Virum, DENMARK
Bjorn, Sara Petersen, Lyngby, DENMARK
Thastrup, Ole, Birkerod, DENMARK
Almholt, Dorthe Christensen, Greve, DENMARK
Praestegaard, Morten, Ballerup, DENMARK

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003187056	A1	20031002	
APPLICATION INFO.:	US 2003-257909	A1	20030313	(10)
	WO 2001-DK264		20010411	

	NUMBER	DATE
PRIORITY INFORMATION:	DK 2000-651	20000417
	DK 2000-849	20000529

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: BIRCH STEWART KOLASCH & BIRCH, PO BOX 747, FALLS CHURCH, VA, 22040-0747
NUMBER OF CLAIMS: 33
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 22 Drawing Page(s)
LINE COUNT: 6071
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 3 OF 11 USPATFULL on STN
TI Fluorescent proteins

AB The present invention provides novel engineered derivatives of green fluorescent protein (**GFP**) which have an amino acid sequence which is modified by amino acid **substitution** compared with the amino acid sequence of wild type Green Fluorescent Protein. The modified GFPs exhibit enhanced fluorescence relative to wtGFP when expressed in non-homologous cells at temperatures above 30° C., and when excited at about 490 nm compared to the parent proteins, i.e. wtGFP. An example of a preferred protein is **F64L**-S175G-**E222G**-**GFP**. The modified GFPs provide a means for detecting **GFP** reporters in mammalian cells at lower levels of expression and/or increased sensitivity relative to wtGFP. This greatly improves the usefulness of fluorescent proteins in studying cellular functions in living cells.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2003:251073 USPATFULL			
TITLE:	Fluorescent proteins			
INVENTOR(S):	Stubbs, Simon Lawrence John, Amersham Buckinghamshire, UNITED KINGDOM Jones, Anne Elizabeth, Amersham Buckinghamshire, UNITED KINGDOM Michael, Nigel Paul, Amersham Buckinghamshire, UNITED KINGDOM Thomas, Nicholas, Amersham Buckinghamshire, UNITED KINGDOM			
	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003175859	A1	20030918	
APPLICATION INFO.:	US 2001-967301	A1	20010928	(9)
	NUMBER		DATE	
PRIORITY INFORMATION:	GB 2001-9858		20010423	
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	APPLICATION			
LEGAL REPRESENTATIVE:	AMERSHAM BIOSCIENCES, PATENT DEPARTMENT, 800 CENTENNIAL AVENUE, PISCATAWAY, NJ, 08855			
NUMBER OF CLAIMS:	24			
EXEMPLARY CLAIM:	1			
NUMBER OF DRAWINGS:	7 Drawing Page(s)			
LINE COUNT:	1284			

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 4 OF 11 USPATFULL on STN

TI Long wavelength engineered fluorescent proteins

AB Engineered fluorescent proteins, nucleic acids encoding them and methods of use are provided.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2003:51221 USPATFULL			
TITLE:	Long wavelength engineered fluorescent proteins			
INVENTOR(S):	Tsien, Roger Y., La Jolla, CA, UNITED STATES Remington, James S., Eugene, OR, UNITED STATES Cubitt, Andrew B., San Diego, CA, UNITED STATES Heim, Roger, Del Mar, CA, UNITED STATES Ormo, Mats F., Huddinge, SWEDEN			
PATENT ASSIGNEE(S):	The Regents of the University of California (U.S. corporation)			
	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003036178	A1	20030220	
APPLICATION INFO.:	US 2002-71976	A1	20020205	(10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1999-465142, filed on 16 Dec 1999, GRANTED, Pat. No. US 6403374 Continuation of Ser. No. US 1997-974737, filed on 19 Nov 1997, GRANTED, Pat. No. US 6077707 Continuation of Ser. No. US 1997-911825, filed on 15 Aug 1997, GRANTED, Pat. No. US 6054321 Continuation-in-part of Ser. No. US 1996-706408, filed on 30 Aug 1996, GRANTED, Pat. No. US 6124128

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: GARY CARY WARE & FRIENDENRICH LLP, 4365 EXECUTIVE DRIVE, SUITE 1600, SAN DIEGO, CA, 92121-2189
NUMBER OF CLAIMS: 1
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 53 Drawing Page(s)
LINE COUNT: 2098
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 5 OF 11 USPATFULL on STN
TI LONG WAVELENGTH ENGINEERED FLUORESCENT PROTEINS
AB Engineered fluorescent proteins, nucleic acids encoding them and methods of use.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:17397 USPATFULL
TITLE: LONG WAVELENGTH ENGINEERED FLUORESCENT PROTEINS
INVENTOR(S): Wachter, Rebekka M., Creswell, OR, UNITED STATES
Remington, S. James, Eugene, OR, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003013149	A1	20030116
	US 6593135	B2	20030715
APPLICATION INFO.:	US 2000-575847	A1	20000519 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1997-974737, filed on 19 Nov 1997, GRANTED, Pat. No. US 6077707 Continuation of Ser. No. US 1997-911825, filed on 15 Aug 1997, GRANTED, Pat. No. US 6054321 Continuation of Ser. No. US 1996-706408, filed on 30 Aug 1996, GRANTED, Pat. No. US 6124128

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Lisa A Haile Ph D, Gray Cary Ware & Freidenrich LLP, 4365 Executive Drive, Suite 1100, San Diego, CA, 92121-2133
NUMBER OF CLAIMS: 187
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 63 Drawing Page(s)
LINE COUNT: 3752
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 6 OF 11 USPATFULL on STN
TI Novel fluorescent proteins
AB A **GFP** with an **F64L** mutation and an **E222G** mutation is provided. This **GFP** has a bigger Stokes shift compared to other GFPs making it very suitable for high throughput

screening due to a better resolution. This **GFP** also has an excitation maximum between the yellow **GFP** and the cyan **GFP** allowing for cleaner band separation when used together with those GFPs.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:314712 USPATFULL		
TITLE:	Novel fluorescent proteins		
INVENTOR(S):	Bjorn, Sara Petersen, Lyngby, DENMARK Pagliaro, Len, Copenhagen K, DENMARK Thastrup, Ole, Birkerod, DENMARK		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002177189	A1	20021128
APPLICATION INFO.:	US 2001-887784	A1	20010619 (9)
	NUMBER		DATE
PRIORITY INFORMATION:	DK 2000-953		20000619
	DK 2001-739		20010510
	US 2000-212681P		20000620 (60)
	US 2001-290170P		20010510 (60)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	BIRCH STEWART KOLASCH & BIRCH, PO BOX 747, FALLS CHURCH, VA, 22040-0747		
NUMBER OF CLAIMS:	19		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	8 Drawing Page(s)		
LINE COUNT:	1225		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 7 OF 11 USPATFULL on STN

TI Long wavelength engineered fluorescent proteins

AB Engineered fluorescent proteins, nucleic acids encoding them and methods of use are provided.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:136818 USPATFULL		
TITLE:	Long wavelength engineered fluorescent proteins		
INVENTOR(S):	Tsien, Roger Y., La Jolla, CA, United States Remington, S. James, Eugene, OR, United States Cubitt, Andrew B., San Diego, CA, United States Heim, Roger, Del Mar, CA, United States Ormo , Mats F., Huddinge, SWEDEN		
PATENT ASSIGNEE(S):	The Regents of the University of California, Oakland, CA, United States (U.S. corporation)		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6403374	B1	20020611
APPLICATION INFO.:	US 1999-465142		19991216 (9)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1997-974737, filed on 19 Nov 1997, now patented, Pat. No. US 6077707 Continuation of Ser. No. US 1997-911825, filed on 15 Aug 1997, now patented, Pat. No. US 6054321 Continuation-in-part of Ser. No. US 1996-706408, filed on 30 Aug 1996, now patented, Pat. No. US 6124128		
	NUMBER		DATE
PRIORITY INFORMATION:	US 1996-24050P		19960816 (60)
DOCUMENT TYPE:	Utility		

FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Nashed, Nashaat T.
LEGAL REPRESENTATIVE: Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
NUMBER OF CLAIMS: 23
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 55 Drawing Figure(s); 53 Drawing Page(s)
LINE COUNT: 2152
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 8 OF 11 USPATFULL on STN
TI Long wavelength engineered fluorescent proteins
AB Engineered fluorescent proteins, nucleic acids encoding them and methods of use.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2000:128162 USPATFULL
TITLE: Long wavelength engineered fluorescent proteins
INVENTOR(S): Tsien, Roger Y., La Jolla, CA, United States
Cubitt, Andrew B., San Diego, CA, United States
Heim, Roger, Del Mar, CA, United States
Ormo, Mats F., Huddinge, Sweden
Remington, S. James, Eugene, OR, United States
PATENT ASSIGNEE(S): The Regents of the University of California, Oakland, CA, United States (U.S. corporation)
Aurora Biosciences, La Jolla, CA, United States (U.S. corporation)
The University of Oregon, Eugene, OR, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6124128		20000926	
APPLICATION INFO.:	US 1996-706408		19960830	(8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Achutamurthy, Ponnathapura
ASSISTANT EXAMINER: Nashed, Nashaat T.
LEGAL REPRESENTATIVE: Fish & Richardson P.C.
NUMBER OF CLAIMS: 37
EXEMPLARY CLAIM: 9
NUMBER OF DRAWINGS: 55 Drawing Figure(s); 53 Drawing Page(s)
LINE COUNT: 1735
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 9 OF 11 USPATFULL on STN
TI Long wavelength engineered fluorescent proteins
AB This invention provides functional engineered fluorescent proteins with varied fluorescence characteristics that can be easily distinguished from currently existing green and blue fluorescent proteins. In one aspect, the invention provides nucleic acids, expression vectors and recombinant host cells comprising nucleotide sequences encoding functional engineered fluorescent proteins comprising aromatic substitutions at position 66 and a folding mutation. In one embodiment the invention provides for fluorescent proteins containing an aromatic **substitution** at Thr 203.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2000:77223 USPATFULL
TITLE: Long wavelength engineered fluorescent proteins
INVENTOR(S): Tsien, Roger Y., La Jolla, CA, United States
Remington, S. James, Eugene, OR, United States
Cubitt, Andrew B., San Diego, CA, United States
Heim, Roger, Del Mar, CA, United States
Ormo, Mats F., Huddinge, Sweden

PATENT ASSIGNEE(S): The Regents of the University of California, Oakland, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6077707		20000620
APPLICATION INFO.:	US 1997-974737		19971119 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1997-911825, filed on 15 Aug 1997 which is a continuation-in-part of Ser. No. US 1996-706408, filed on 30 Aug 1996

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Nashed, Nashaat
LEGAL REPRESENTATIVE: Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
NUMBER OF CLAIMS: 17
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 53 Drawing Figure(s); 53 Drawing Page(s)
LINE COUNT: 2162
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 10 OF 11 USPATFULL on STN
TI Long wavelength engineered fluorescent proteins
AB This invention provides functional engineered fluorescent proteins with varied fluorescence characteristics that can be easily distinguished from currently existing green and blue fluorescent proteins. In one embodiment the invention provides for the three dimensional structure and atomic coordinates of an Aequorea green fluorescent protein and methods for their use. In one embodiment, this invention provides a computational method of modeling the three dimensional structure of any other fluorescent protein based on the three dimensional structure of an Aequorea green fluorescent protein.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2000:50571 USPATFULL
TITLE: Long wavelength engineered fluorescent proteins
INVENTOR(S): Tsien, Roger Y., La Jolla, CA, United States
Remington, S. James, Eugene, OR, United States
Cubitt, Andrew B., San Diego, CA, United States
Heim, Roger, Del Mar, CA, United States
Ormo, Mats F., Huddinge, Sweden
PATENT ASSIGNEE(S): The Regents of the University of California, Oakland, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6054321		20000425
APPLICATION INFO.:	US 1997-911825		19970815 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1996-706408, filed on 30 Aug 1996

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Nashed, Nashaat
LEGAL REPRESENTATIVE: Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
NUMBER OF CLAIMS: 15
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 36 Drawing Figure(s); 53 Drawing Page(s)

LINE COUNT: 2254
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 11 OF 11 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI New long wavelength engineered fluorescent proteins, useful as markers for gene expression, tracers of cell lineage or as fusion tags to monitor protein localization, or in detection assays, e.g. immunoassays or hybridization assays.
AN 2002-083084 [11] WPIDS
CR 1998-159454 [14]
AB WO 200190147 A UPAB: 20030729
NOVELTY - A functional engineered fluorescent protein (I), whose is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (A-**GFP**) having a 238 residue amino acid sequence (I-a), fully defined in the specification, is new.

DETAILED DESCRIPTION - A functional engineered fluorescent protein (I), whose is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (A-**GFP**) having a 238 residue amino acid sequence (I-a), fully defined in the specification, is new. (I) has an amino acid sequence that:

(a) differs from (I-a) by at least the amino acid **substitution** T203X; or

(b) differs from (I-a) by at least an amino acid **substitution** at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, 1167, Q183, N185, L220, E222 (not **E222G**) or V224.

(I) has a different fluorescent property than A-**GFP**.

X = an aromatic amino acid selected from H, Y, W or F.

INDEPENDENT CLAIMS are also included for the following:

(1) nucleic acid molecules comprising a nucleotide sequence encoding (I);

(2) expression vectors comprising expression control sequences operatively linked to the nucleic acid molecule comprising a sequence encoding (I);

(3) host cells comprising:

(a) recombinant host cells comprising the expression vectors; or

(b) (I) whose amino acid sequence differs from (I-a) by at least one first **substitution** at position T203, and at least one second **substitution** at position H148;

(4) fluorescently labeled antibodies comprising antibodies coupled to (I);

(5) nucleic acid molecules comprising nucleotide sequences encoding the antibodies fused to nucleotide sequences encoding (I);

(6) fluorescently labeled nucleic acid probes comprising a nucleic acid probes coupled to (I);

(7) determining if a mixture contains a target comprising:

(a) contacting the mixture with the fluorescently labeled probes; and

(b) determining if the target has bound to the probe;

(8) engineering (I), which has a fluorescent property different from A-**GFP**;

(9) producing fluorescence resonance energy transfers;

(10) protein comprising (I), where the crystal diffracts with at least a 2.0-3.0 Angstrom resolution;

(11) a computational method of designing a fluorescent protein;

(12) a computational method of modeling the three dimensional structure of a fluorescent protein by determining a three dimensional relationship between at least two atoms listed in the atomic coordinates fully described in the specification;

(13) a device comprising a storage device and, stored in the device, at least 10 atomic coordinates selected from those atomic coordinates fully listed in the specification;

(14) identifying a test chemical, comprising:

(a) contacting a test chemical with a sample containing a biological entity labeled with (I) or a polynucleotide encoding (I); and

(b) detecting fluorescence of (I);

(15) determining the presence of an anion of interest in a sample, comprising:

(a) introducing (I) into a sample; and

(b) determining the fluorescence of (I); and

(16) screening the effects of test compounds on ion channel activity.

USE - (I) is useful as markers for gene expression, tracers of cell lineage or as fusion tags to monitor protein localization within living cells. (I) is particularly useful for coupling engineered fluorescent proteins to antibodies, nucleic acids or other receptors for use in detection assays, e.g. immunoassays or hybridization assays. (I) is also useful for tracking the movement of proteins in cells, or in systems for detecting induction of transcription. (I) is particularly useful for the simultaneous measurement of two or more processes within cells and is also useful as fluorescent energy donors or acceptors, as well as biosensors for detecting anions. (I) is also useful in fluorescence resonance energy transfer (FRET). The crystal structure of the green fluorescent protein is useful for designing mutants having altered fluorescent characteristics. This is particularly useful in identifying amino acids whose substitution alters fluorescent properties of the protein. The crystal structure of the green fluorescent protein is also useful for designing mutants having altered anion binding characteristics. This is particularly useful for identifying amino acids whose substitution alters the specificity and affinity of the binding site to various anions, and for monitoring anion binding and therefore the concentration of the anion.

ADVANTAGE - The present engineered fluorescent protein has varied fluorescent properties and has the ability to respond to ion concentrations via a change in fluorescent characteristics. The functional engineered fluorescent proteins with varied fluorescent characteristics can be easily distinguished from currently existing green and blue fluorescent proteins. The engineered fluorescent proteins enable the simultaneous measurement of two or more processes within cells and can be used as fluorescent energy donors or acceptors, as well as biosensors for detecting anions. The present engineered fluorescent proteins are particularly useful because photodynamic toxicity and auto-fluorescence of cells are significantly reduced at longer wavelengths. In particular, the introduction of the substitution T203X, where X is an aromatic amino acid, results in an increase in the excitation and emission wavelength maxima of Aequorea-related fluorescent proteins. Another primary advantage of fluorescent protein fusion proteins is that they are prepared by normal protein biosynthesis, thus completely avoiding organic synthesis and the requirement for customized unnatural amino acid analogs.

Dwg.0/15

ACCESSION NUMBER: 2002-083084 [11] WPIDS
CROSS REFERENCE: 1998-159454 [14]
DOC. NO. CPI: C2002-025219

TITLE: New long wavelength engineered fluorescent proteins, useful as markers for gene expression, tracers of cell lineage or as fusion tags to monitor protein localization, or in detection assays, e.g. immunoassays or hybridization assays.

DERWENT CLASS: B04 D16

INVENTOR(S): REMINGTON, S J; WACHTER, R; WACHTER, R M
PATENT ASSIGNEE(S): (UYOR-N) UNIV OREGON HEALTH SCI; (UYOR-N) UNIV OREGON STATE

COUNTRY COUNT: 96
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001090147 A2		20011129	(200211)*	EN	181

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ

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          LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD
          SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
     AU 2001063269 A  20011203 (200221)
     EP 1285065    A2 20030226 (200319)  EN
        R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
          RO SE SI TR
     US 6593135    B2 20030715 (200348)

APPLICATION DETAILS:

     PATENT NO   KIND                  APPLICATION      DATE
     ----------------------------------------------------------
     WO 2001090147 A2                  WO 2001-US16149  20010517
     AU 2001063269 A                   AU 2001-63269    20010517
     EP 1285065    A2                  EP 2001-937550   20010517
                                       WO 2001-US16149  20010517
     US 6593135    B2 Provisional      US 1996-24050P   19960816
                      CIP of           US 1996-706408   19960830
                      Cont of          US 1997-911825   19970815
                      CIP of           US 1997-974737   19971119
                                       US 2000-575847   20000519

FILING DETAILS:

     PATENT NO   KIND                  PATENT NO
     ----------------------------------------------
     AU 2001063269 A  Based on         WO 2001090147
     EP 1285065    A2 Based on         WO 2001090147
     US 6593135    B2 Cont of          US 6054321
                      CIP of           US 6077707
                      CIP of           US 6124128

PRIORITY APPLN. INFO: US 2000-575847   20000519; US 1996-24050P
                      19960816; US 1996-706408   19960830; US
                      1997-911825   19970815; US 1997-974737   19971119


=> d his

     (FILE 'HOME' ENTERED AT 15:48:20 ON 19 FEB 2004)

     FILE 'MEDLINE, USPATFULL, DGENE, EMBASE, WPIDS, FSTA, JICST-EPLUS,
     BIOSIS, HCAPLUS' ENTERED AT 15:48:47 ON 19 FEB 2004
L1         43171 S GFP
L2             5 S GFP ANALOGUE
L3          1458 S CHROMOPHORE AND L1
L4           369 S L3 AND SUBSTITUTION
L5            13 S L4 AND POSITION 64
L6            15 S L4 AND E222G
L7            51 S L4 AND F64L
L8            11 S L7 AND L6

=> s aequoria victorea or renilla
L9         2813 AEQUORIA VICTOREA OR RENILLA

=> s l9 and l1
L10        1187 L9 AND L1

=> s l8 and l10
L11           8 L8 AND L10

=> d l11 ti abs ibib tot

L11  ANSWER 1 OF 8  USPATFULL on STN
```

TI Long wavelength engineered fluorescent proteins
AB Engineered fluorescent proteins, nucleic acids encoding them and methods of use.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2004:18826 USPATFULL
TITLE:	Long wavelength engineered fluorescent proteins
INVENTOR(S):	Wachter, Rebekka M., Creswell, OR, UNITED STATES Remington, S. James, Eugene, OR, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2004014128	A1	20040122
APPLICATION INFO.:	US 2003-620099	A1	20030714 (10)

RELATED APPLN. INFO.: Division of Ser. No. US 2000-575847, filed on 19 May 2000, GRANTED, Pat. No. US 6593135 Continuation-in-part of Ser. No. US 1997-974737, filed on 19 Nov 1997, GRANTED, Pat. No. US 6077707 Continuation of Ser. No. US 1997-911825, filed on 15 Aug 1997, GRANTED, Pat. No. US 6054321 Continuation-in-part of Ser. No. US 1996-706408, filed on 30 Aug 1996, GRANTED, Pat. No. US 6124128

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	Lisa A. Haile, J.D., Ph.D., GRAY CARY WARE & FREIDENRICH LLP, Suite 1100, 4365 Executive Drive, San Diego, CA, 92121-2133
NUMBER OF CLAIMS:	187
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	62 Drawing Page(s)
LINE COUNT:	3919

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 2 OF 8 USPATFULL on STN
TI Long wavelength engineered fluorescent proteins
AB Engineered fluorescent proteins, nucleic acids encoding them and methods of use are provided.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2003:51221 USPATFULL
TITLE:	Long wavelength engineered fluorescent proteins
INVENTOR(S):	Tsien, Roger Y., La Jolla, CA, UNITED STATES Remington, James S., Eugene, OR, UNITED STATES Cubitt, Andrew B., San Diego, CA, UNITED STATES Heim, Roger, Del Mar, CA, UNITED STATES Ormo, Mats F., Huddinge, SWEDEN
PATENT ASSIGNEE(S):	The Regents of the University of California (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003036178	A1	20030220
APPLICATION INFO.:	US 2002-71976	A1	20020205 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1999-465142, filed on 16 Dec 1999, GRANTED, Pat. No. US 6403374 Continuation of Ser. No. US 1997-974737, filed on 19 Nov 1997, GRANTED, Pat. No. US 6077707 Continuation of Ser. No. US 1997-911825, filed on 15 Aug 1997, GRANTED, Pat. No. US 6054321 Continuation-in-part of Ser. No. US 1996-706408, filed on 30 Aug 1996, GRANTED, Pat. No. US

6124128

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: GARY CARY WARE & FRIENDENRICH LLP, 4365 EXECUTIVE DRIVE, SUITE 1600, SAN DIEGO, CA, 92121-2189
NUMBER OF CLAIMS: 1
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 53 Drawing Page(s)
LINE COUNT: 2098
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 3 OF 8 USPATFULL on STN
TI LONG WAVELENGTH ENGINEERED FLUORESCENT PROTEINS
AB Engineered fluorescent proteins, nucleic acids encoding them and methods of use.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:17397 USPATFULL
TITLE: LONG WAVELENGTH ENGINEERED FLUORESCENT PROTEINS
INVENTOR(S): Wachter, Rebekka M., Creswell, OR, UNITED STATES
Remington, S. James, Eugene, OR, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003013149	A1	20030116
	US 6593135	B2	20030715
APPLICATION INFO.:	US 2000-575847	A1	20000519 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1997-974737, filed on 19 Nov 1997, GRANTED, Pat. No. US 6077707 Continuation of Ser. No. US 1997-911825, filed on 15 Aug 1997, GRANTED, Pat. No. US 6054321 Continuation of Ser. No. US 1996-706408, filed on 30 Aug 1996, GRANTED, Pat. No. US 6124128

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Lisa A Haile Ph D, Gray Cary Ware & Freidenrich LLP, 4365 Executive Drive, Suite 1100, San Diego, CA, 92121-2133
NUMBER OF CLAIMS: 187
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 63 Drawing Page(s)
LINE COUNT: 3752
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 4 OF 8 USPATFULL on STN
TI Novel fluorescent proteins
AB A **GFP** with an **F64L** mutation and an **E222G** mutation is provided. This **GFP** has a bigger Stokes shift compared to other GFPs making it very suitable for high throughput screening due to a better resolution. This **GFP** also has an excitation maximum between the yellow **GFP** and the cyan **GFP** allowing for cleaner band separation when used together with those GFPs.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:314712 USPATFULL

TITLE: Novel fluorescent proteins
INVENTOR(S): Bjorn, Sara Petersen, Lyngby, DENMARK
Pagliaro, Len, Copenhagen K, DENMARK
Thastrup, Ole, Birkerod, DENMARK

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002177189	A1	20021128
APPLICATION INFO.:	US 2001-887784	A1	20010619 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	DK 2000-953	20000619
	DK 2001-739	20010510
	US 2000-212681P	20000620 (60)
	US 2001-290170P	20010510 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: BIRCH STEWART KOLASCH & BIRCH, PO BOX 747, FALLS CHURCH, VA, 22040-0747
NUMBER OF CLAIMS: 19
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 8 Drawing Page(s)
LINE COUNT: 1225
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 5 OF 8 USPATFULL on STN
TI Long wavelength engineered fluorescent proteins
AB Engineered fluorescent proteins, nucleic acids encoding them and methods of use are provided.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:136818 USPATFULL
TITLE: Long wavelength engineered fluorescent proteins
INVENTOR(S): Tsien, Roger Y., La Jolla, CA, United States
Remington, S. James, Eugene, OR, United States
Cubitt, Andrew B., San Diego, CA, United States
Heim, Roger, Del Mar, CA, United States
Ormo , Mats F., Huddinge, SWEDEN
PATENT ASSIGNEE(S): The Regents of the University of California, Oakland, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6403374	B1	20020611
APPLICATION INFO.:	US 1999-465142		19991216 (9)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1997-974737, filed on 19 Nov 1997, now patented, Pat. No. US 6077707 Continuation of Ser. No. US 1997-911825, filed on 15 Aug 1997, now patented, Pat. No. US 6054321 Continuation-in-part of Ser. No. US 1996-706408, filed on 30 Aug 1996, now patented, Pat. No. US 6124128		

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Nashed, Nashaat T.
LEGAL REPRESENTATIVE: Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
NUMBER OF CLAIMS: 23
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 55 Drawing Figure(s); 53 Drawing Page(s)
LINE COUNT: 2152

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 6 OF 8 USPATFULL on STN
TI Long wavelength engineered fluorescent proteins
AB Engineered fluorescent proteins, nucleic acids encoding them and methods of use.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2000:128162 USPATFULL
TITLE:	Long wavelength engineered fluorescent proteins
INVENTOR(S):	Tsien, Roger Y., La Jolla, CA, United States Cubitt, Andrew B., San Diego, CA, United States Heim, Roger, Del Mar, CA, United States Ormo, Mats F., Huddinge, Sweden Remington, S. James, Eugene, OR, United States
PATENT ASSIGNEE(S):	The Regents of the University of California, Oakland, CA, United States (U.S. corporation) Aurora Biosciences, La Jolla, CA, United States (U.S. corporation) The University of Oregon, Eugene, OR, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6124128		20000926	
APPLICATION INFO.:	US 1996-706408		19960830	(8)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	Granted
PRIMARY EXAMINER:	Achutamurthy, Ponnathapura
ASSISTANT EXAMINER:	Nashed, Nashaat T.
LEGAL REPRESENTATIVE:	Fish & Richardson P.C.
NUMBER OF CLAIMS:	37
EXEMPLARY CLAIM:	9
NUMBER OF DRAWINGS:	55 Drawing Figure(s); 53 Drawing Page(s)
LINE COUNT:	1735

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 7 OF 8 USPATFULL on STN
TI Long wavelength engineered fluorescent proteins
AB This invention provides functional engineered fluorescent proteins with varied fluorescence characteristics that can be easily distinguished from currently existing green and blue fluorescent proteins. In one aspect, the invention provides nucleic acids, expression vectors and recombinant host cells comprising nucleotide sequences encoding functional engineered fluorescent proteins comprising aromatic substitutions at position 66 and a folding mutation. In one embodiment the invention provides for fluorescent proteins containing an aromatic **substitution** at Thr 203.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2000:77223 USPATFULL
TITLE:	Long wavelength engineered fluorescent proteins
INVENTOR(S):	Tsien, Roger Y., La Jolla, CA, United States Remington, S. James, Eugene, OR, United States Cubitt, Andrew B., San Diego, CA, United States Heim, Roger, Del Mar, CA, United States Ormo, Mats F., Huddinge, Sweden
PATENT ASSIGNEE(S):	The Regents of the University of California, Oakland, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6077707		20000620	
APPLICATION INFO.:	US 1997-974737		19971119	(8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1997-911825, filed on 15 Aug 1997 which is a continuation-in-part of Ser. No. US 1996-706408, filed on 30 Aug 1996

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Nashed, Nashaat
LEGAL REPRESENTATIVE: Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
NUMBER OF CLAIMS: 17
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 53 Drawing Figure(s); 53 Drawing Page(s)
LINE COUNT: 2162
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 8 OF 8 USPATFULL on STN
TI Long wavelength engineered fluorescent proteins
AB This invention provides functional engineered fluorescent proteins with varied fluorescence characteristics that can be easily distinguished from currently existing green and blue fluorescent proteins. In one embodiment the invention provides for the three dimensional structure and atomic coordinates of an Aequorea green fluorescent protein and methods for their use. In one embodiment, this invention provides a computational method of modeling the three dimensional structure of any other fluorescent protein based on the three dimensional structure of an Aequorea green fluorescent protein.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2000:50571 USPATFULL
TITLE: Long wavelength engineered fluorescent proteins
INVENTOR(S): Tsien, Roger Y., La Jolla, CA, United States
Remington, S. James, Eugene, OR, United States
Cubitt, Andrew B., San Diego, CA, United States
Heim, Roger, Del Mar, CA, United States
Ormo, Mats F., Huddinge, Sweden
PATENT ASSIGNEE(S): The Regents of the University of California, Oakland, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6054321		20000425
APPLICATION INFO.:	US 1997-911825		19970815 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1996-706408, filed on 30 Aug 1996

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-24050P	19960816 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Nashed, Nashaat
LEGAL REPRESENTATIVE: Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
NUMBER OF CLAIMS: 15
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 36 Drawing Figure(s); 53 Drawing Page(s)
LINE COUNT: 2254
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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DB=USPT; PLUR=YES; OP=OR			
L7	L6 and E222G	5	L7
L6	L5 and F64L	51	L6
L5	L4 and mutation	1607	L5
L4	L3 and l2	2114	L4
L3	GFP analogue	302944	L3
L2	GFP	2114	L2
L1	chromophore	8895	L1

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Search Results - Record(s) 1 through 5 of 5 returned.

☐ 1. Document ID: US 6593135 B2

L7: Entry 1 of 5 File: USPT Jul 15, 2003

US-PAT-NO: 6593135
DOCUMENT-IDENTIFIER: US 6593135 B2

TITLE: Long wavelength engineered fluorescent proteins

DATE-ISSUED: July 15, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wachter; Rebekka M.	Creswell	OR		
Remington; S. James	Eugene	OR		

US-CL-CURRENT: 435/325; 435/252.3, 435/252.33, 435/254.11, 435/320.1, 435/410, 536/23.1, 536/23.4, 536/23.6

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw De

☐ 2. Document ID: US 6403374 B1

L7: Entry 2 of 5 File: USPT Jun 11, 2002

US-PAT-NO: 6403374
DOCUMENT-IDENTIFIER: US 6403374 B1
** See image for Certificate of Correction **

TITLE: Long wavelength engineered fluorescent proteins

DATE-ISSUED: June 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Remington; S. James	Eugene	OR		
Cubitt; Andrew B.	San Diego	CA		
Heim; Roger	Del Mar	CA		
Ormo ; Mats F.	Huddinge			SE

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US-CL-CURRENT: 435/325; 435/252.3, 435/252.33, 435/254.11, 435/320.1, 435/410, 536/23.1, 536/23.4, 536/23.6

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 3. Document ID: US 6124128 A

L7: Entry 3 of 5 File: USPT Sep 26, 2000

US-PAT-NO: 6124128
DOCUMENT-IDENTIFIER: US 6124128 A

TITLE: Long wavelength engineered fluorescent proteins

DATE-ISSUED: September 26, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Cubitt; Andrew B.	San Diego	CA		
Heim; Roger	Del Mar	CA		
Ormo; Mats F.	Huddinge			SE
Remington; S. James	Eugene	OR		

US-CL-CURRENT: 435/252.33; 435/252.3, 435/320.1, 536/23.1, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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☐ 4. Document ID: US 6077707 A

L7: Entry 4 of 5 File: USPT Jun 20, 2000

US-PAT-NO: 6077707
DOCUMENT-IDENTIFIER: US 6077707 A

TITLE: Long wavelength engineered fluorescent proteins

DATE-ISSUED: June 20, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Remington; S. James	Eugene	OR		
Cubitt; Andrew B.	San Diego	CA		
Heim; Roger	Del Mar	CA		
Ormo; Mats F.	Huddinge			SE

US-CL-CURRENT: 435/325; 435/252.3, 435/252.33, 435/254.11, 435/320.1, 435/410, 435/69.1, 530/350, 536/23.1, 536/23.5

h e b b g e e e f e ef b e

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw De

☐ 5. Document ID: US 6054321 A

L7: Entry 5 of 5 File: USPT Apr 25, 2000

US-PAT-NO: 6054321
DOCUMENT-IDENTIFIER: US 6054321 A

TITLE: Long wavelength engineered fluorescent proteins

DATE-ISSUED: April 25, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsien; Roger Y.	La Jolla	CA		
Remington; S. James	Eugene	OR		
Cubitt; Andrew B.	San Diego	CA		
Heim; Roger	Del Mar	CA		
Ormo; Mats F.	Huddinge			SE

US-CL-CURRENT: 436/86; 530/350, 702/19, 702/22

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw De

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Terms	Documents
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L7: Entry 1 of 5 F64L E222 N or Q not G File: USPT Jul 15, 2003

DOCUMENT-IDENTIFIER: US 6593135 B2
TITLE: Long wavelength engineered fluorescent proteins

Brief Summary Text (2):
Fluorescent molecules are attractive as reporter molecules in many assay systems because of their high sensitivity and ease of quantification. Recently, fluorescent proteins have been the focus of much attention because they can be produced in vivo by biological systems, and can be used to trace intracellular events without the need to be introduced into the cell through microinjection or permeablization. The green fluorescent protein of Aequorea victoria is particularly interesting as a fluorescent protein. A cDNA for the protein has been cloned. (D. C. Prasher et al., "Primary structure of the Aequorea victoria green-fluorescent protein," Gene (1992) 111:229-33.) Not only can the primary amino acid sequence of the protein be expressed from the cDNA, but the expressed protein can fluoresce. This indicates that the protein can undergo the cyclization and oxidation believed to be necessary for fluorescence. Aequorea green fluorescent protein ("GFP") is a stable, proteolysis-resistant single chain of 238 residues and has two absorption maxima at around 395 and 475 nm. The relative amplitudes of these two peaks is sensitive to environmental factors (W. W. Ward. Bioluminescence and Chemiluminescence (M. A. DeLuca and W. D. McElroy, eds) Academic Press pp. 235-242 (1981); W. W. Ward & S. H. Bokman Biochemistry 21:4535-4540 (1982); W. W. Ward et al. Photochem. Photobiol. 35:803-808 (1982)) and illumination history (A. B. Cubitt et al. Trends Biochem. Sci. 20:448-455 (1995)), presumably reflecting two or more ground states. Excitation at the primary absorption peak of 395 nm yields an emission maximum at 508 nm with a quantum yield of 0.72-0.85 (O. Shimomura and F. H. Johnson J. Cell. Comp. Physiol. 59:223 (1962); J. G. Morin and J. W. Hastings, J. Cell. Physiol. 77:313 (1971); H. Morise et al. Biochemistry 13:2656 (1974); W. W. Ward Photochem. Photobiol. Reviews (Smith, K. C. ed.) 4:1 (1979); A. B. Cubitt et al. Trends Biochem. Sci. 20:448-455 (1995); D. C. Prasher Trends Genet. 11:320-323 (1995); M. Chalfie Photochem. Photobiol. 62:651-656 (1995); W. W. Ward. Bioluminescence and Chemiluminescence (M. A. DeLuca and W. D. McElroy, eds) Academic Press pp. 235-242 (1981); W. W. Ward & S. H. Bokman Biochemistry 21:4535-4540 (1982); W. W. Ward et al. Photochem. Photobiol. 35:803-808 (1982)). The fluorophore results from the autocatalytic cyclization of the polypeptide backbone between residues Ser.sup.65 and Gly.sup.67 and oxidation of the -.beta. bond of Tyr.sup.66 (A. B. Cubitt et al. Trends Biochem. Sci. 20:448-455 (1995); C. W. Cody et al. Biochemistry 32:1212-1218 (1993); R. Heim et al. Proc. Natl. Acad Sci. USA 91:12501-12504 (1994)). Mutation of Ser.sup.65 to Thr (S65T) simplifies the excitation spectrum to a single peak at 488 nm of enhanced amplitude (R. Heim et al. Nature 373:664-665 (1995)), which no longer gives signs of conformational isomers (A. B. Cubitt et al. Trends Biochem. Sci. 20:448-455 (1995)).

Brief Summary Text (3):
Fluorescent proteins have been used as markers of gene expression, tracers of cell lineage and as fusion tags to monitor protein localization within living cells. (M. Chalfie et al., "Green fluorescent protein as a marker for gene expression," Science 263:802-805; A. B. Cubitt et al., "Understanding, improving and using green fluorescent proteins," TIBS 20, November 1995, pp.448-455. U.S. Pat. No. 5,491,084, M. Chalfie and D. Prasher. Furthermore, engineered versions of Aequorea green fluorescent protein have been identified that exhibit altered fluorescence

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characteristics, including altered excitation and emission maxima, as well as excitation and emission spectra of different shapes. (R. Heim et al., "Wavelength mutations and posttranslational autoxidation of green fluorescent protein," Proc. Natl. Acad. Sci. USA, (1994) 91:12501-04; R. Heim et al., "Improved green fluorescence," Nature (1995) 373:663-665.)

Brief Summary Text (4):
A second class of applications rely on GFP as a specific indicator of some cellular property, and hence depend on the particular spectral characteristics of the variant employed. For recent reviews on GFP variants and their applications, see (Palm & Wlodawer, 1999; Tsien, 1998), and for a review volume on specialized applications, see (Sullivan & Kay, 1999). Biosensor applications include the use of differently colored GFPs for fluorescence resonance energy transfer (FRET) to monitor protein-protein interactions (Heim, 1999) or Ca2+ concentrations (Miyawaki et al., 1999), and receptor insertions within GFP surface loops to monitor ligand binding (Baird et al., 1999; Doi & Yanagawa, 1999).

Brief Summary Text (5):
The fluorescence emission of a number of variants is highly sensitive to the acidity of the environment (Elsliger et al., 1999; Wachter et al., 1998). Hence, one particularly successful application of green fluorescent protein (GFP) as a visual reporter in live cells has been the determination of organelle or cytosol pH (Kneen et al., 1998; Llopis et al., 1998; Miesenbock et al., 1998; Robey et al., 1998). The two chromophore charge states have been found to be relevant to the pH sensitivity of the intact protein, and have been characterized crystallographically in terms of conformational changes in the vicinity of the phenolic end (Elsliger et al., 1999), and spectroscopically using Raman studies (Bell et al., 2000). The neutral form of the chromophore, band A, absorbs around 400 nm in most variants, whereas the chromophore anion with the phenolic end deprotonated (band B) absorbs in the blue to green, depending on the particular mutations in the vicinity of the chromophore. WT GFP exhibits spectral characteristics that are consistent with two ground states characterized by a combination of bands A and B, the ratio of which is relatively invariant between pH 6 and 10 (Palm & Wlodawer, 1999; Ward et al., 1982). It has been suggested that an internal equilibrium exists where a proton is shared between the chromophore phenolate and the carboxylate of Glu222 over a broad range of pH (Brejc et al., 1997; Palm et al., 1997). Recent electrostatic calculations support this model (Scharnagl et al., 1999), and estimate the theoretical pK.sub.a for complete chromophore deprotonation to be about 13, consistent with the observation of a doubling of emission intensity at pH 11-12 (Bokman & Ward, 1981; Palm & Wlodawer, 1999).

Brief Summary Text (6):
In contrast to WT GFP, the chromophore of most variants titrates with a single pK.sub.a. The color emission and the chromophore pK.sub.a are strongly modulated by the protein surroundings (Llopis et al., 1998). Glu222 is completely conserved among GFP homologs (Matz et al., 1999), and its substitution by a glutamine has been shown to dramatically reduce efficiency of chromophore generation (Elsliger et al., 1999). Protonation of Glu222 in S65T and in GFPs containing the T203Y mutation (YFPs) is generally thought to be responsible for lowering the chromophore pKa from that of WT to about 5.9 in GFP S65T (Elsliger et al., 1999; Kneen et al., 1998), and 5.2-5.4 in YFP (GFP S65G/V68L/S72A/T203Y) (Ormo et al., 1996; Wachter & Remington, 1999). In the YFPs, it is thought that the crystallographically identified stacking interaction of the chromophore with Tyr203 is largely responsible for the spectral red-shift (Wachter et al., 1998).

Brief Summary Text (7):
Unlike other variants, we have discovered that the YFP chromophore pK.sub.a shows a strong dependence on the concentration of certain small anions such as chloride (Wachter & Remington, 1999), and increases in pK.sub.a from about 5.2 to 7.0 in the presence of 140 mM NaCl (Elsliger et al., 1999). This sensitivity can be exploited

to enable the creation of novel GFPs as biosensors to measure ions present both in the cytoplasm or in cellular compartments (Wachter & Remington, 1999) within living cells. The present invention includes the creation and use of novel GFP variants that permit the fluorescent measurement of a variety of ions, including halides such as chloride and iodide. These properties add variety and utility to the arsenal of biologically based fluorescent indicators. There is a need for engineered fluorescent proteins with varied fluorescent properties and with the ability to respond to ion concentrations via a change in fluorescence characteristics.

Drawing Description Text (2):
FIGS. 1A-1B. (A) Schematic drawing of the backbone of GFP produced by Molscript (J. P. Kraulis, J. Appl. Cryst., 24:946 (1991)). The chromophore is shown as a ball and stick model. (B) Schematic drawing of the overall fold of GFP. Approximate residue numbers mark the beginning and ending of the secondary structure elements.

Detailed Description Text (4):
In one aspect this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at T203 and, in particular, T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than Aequorea green fluorescent protein. In one embodiment, the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S651. In another embodiment, the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S65G/V68L/Q69K/S72A/T203Y; S72A/S65G/V68L/T203Y; S65G/S72A/T203Y S65G/S72A/T203W. In another embodiment, the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W. In another embodiment, the amino acid sequence further comprises a mutation from Table A. In another embodiment, the amino acid sequence further comprises a folding mutation. In another embodiment, the nucleotide sequence encoding the protein differs from the nucleotide sequence of SEQ ID NO:1 by the substitution of at least one codon by a preferred mammalian codon. In another embodiment, the nucleic acid molecule encodes a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

Detailed Description Text (5):
In another aspect, this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, 1167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than Aequorea green fluorescent protein. In one embodiment, amino acid substitution is: L42X, wherein X is selected from C, F, H, W and Y, V61X, wherein X is selected from F, Y, H and C, T62X, wherein X is selected from A, V, F, S, D, N, Q, Y, H and C, V68X, wherein X is selected from F, Y and H, Q69X, wherein X is selected from K, R, E and G, Q94X, wherein X is selected from D, E, H, K and N, N121X, wherein X is selected from F, H, W and Y, Y145X, wherein X is selected from W, C, F, L, E, H, K and Q, H148X, wherein X is selected from F, Y, N, K, Q and R, V150X, wherein X is selected from F, Y and H, F165X, wherein X is selected from H, Q, W and Y,

Detailed Description Text (9):
In another aspect, this invention provides a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from

SEQ ID NO:2 by at least the amino acid substitution at T203, and in particular, T203X, wherein X is an aromatic amino acid selected from H, Y, W or F, said functional engineered fluorescent protein having a different fluorescent property than Aequorea green fluorescent protein. In one embodiment, the amino acid sequence further comprises a substitution at S65, wherein the substitution is selected from S65G, S65T, S65A, S65L, S65C, S65V and S65I. In another embodiment, the amino acid sequence differs by no more than the substitutions S65T/T203H; S65T/T203Y; S72A/F64L/S65G/T203Y; S72A/S65G/V68L/T203Y; S65G/V68L/Q69K/S72A/T203Y; S65G/S72A/T203Y; or S65G/S72A/T203W. In another embodiment, the amino acid sequence further comprises a substitution at Y66, wherein the substitution is selected from Y66H, Y66F, and Y66W. In another embodiment, the amino acid sequence further comprises a folding mutation. In another embodiment, the engineered fluorescent protein is part of a fusion protein wherein the fusion protein comprises a polypeptide of interest and the functional engineered fluorescent protein.

Detailed Description Text (31):
"Nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and, unless otherwise limited, encompasses known analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides. It will be understood that when a nucleic acid molecule is represented by a DNA sequence, this also includes RNA molecules having the corresponding RNA sequence in which "U" replaces "T."

Detailed Description Text (48):
The terms "polypeptide" and "protein" refers to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The term "recombinant protein" refers to a protein that is produced by expression of a nucleotide sequence encoding the amino acid sequence of the protein from a recombinant DNA molecule.

Detailed Description Text (63):
The term "fluorescent property" refers to the molar extinction coefficient at an appropriate excitation wavelength, the fluorescence quantum efficiency, the shape of the excitation spectrum or emission spectrum, the excitation wavelength maximum and emission wavelength maximum, the ratio of excitation amplitudes at two different wavelengths, the ratio of emission amplitudes at two different wavelengths, the excited state lifetime, or the fluorescence anisotropy. A measurable difference in any one of these properties between wild-type Aequorea GFP and the mutant form is useful. A measurable difference can be determined by determining the amount of any quantitative fluorescent property, e.g., the amount of fluorescence at a particular wavelength, or the integral of fluorescence over the emission spectrum. Determining ratios of excitation amplitude or emission amplitude at two different wavelengths ("excitation amplitude ratioing" and "emission amplitude ratioing", respectively) are particularly advantageous because the ratioing process provides an internal reference and cancels out variations in the absolute brightness of the excitation source, the sensitivity of the detector, and light scattering or quenching by the sample.

Detailed Description Text (66):
As used herein, the term "fluorescent protein" refers to any protein capable of fluorescence when excited with appropriate electromagnetic radiation. This includes fluorescent proteins whose amino acid sequences are either naturally occurring or engineered (i.e., analogs or mutants). Many cnidarians use green fluorescent proteins ("GFPs") as energy-transfer acceptors in bioluminescence. A "green fluorescent protein," as used herein, is a protein that fluoresces green light. Similarly, "blue fluorescent proteins" fluoresce blue light and "red fluorescent proteins" fluoresce red light. GFPs have been isolated from the Pacific Northwest jellyfish, Aequorea victoria, the sea pansy, Renilla reniformis, and Phialidium

gregarium. W. W. Ward et al., Photochem. Photobiol., 35:803-808 (1982); L. D. Levine et al., Comp. Biochem. Physiol., 72B:77-85 (1982).

Detailed Description Text (67):
A variety of Aequorea-related fluorescent proteins having useful excitation and emission spectra have been engineered by modifying the amino acid sequence of a naturally occurring GFP from Aequorea victoria. (D. C. Prasher et al., Gene, 111:229-233 (1992); R. Heim et al., Proc. Natl. Acad. Sci., USA, 91:12501-04 (1994); U.S. patent application Ser. No. 08/337,915, filed Nov. 10, 1994; International application PCT/US95/14692, filed Nov. 10, 1995.)

Detailed Description Text (69):
Aequorea-related fluorescent proteins include, for example and without limitation, wild-type (native) Aequorea victoria GFP (D. C. Prasher et al., "Primary structure of the Aequorea victoria green fluorescent protein," Gene, (1992) 111:229-33), whose nucleotide sequence (SEQ ID NO: 1) and deduced amino acid sequence (SEQ ID NO:2) are presented in FIG. 3; allelic variants of this sequence, e.g., Q80R, which has the glutamine residue at position 80 substituted with arginine (M. Chalfie et al., Science, (1994) 263:802-805); those engineered Aequorea-related fluorescent proteins described herein, e.g., in Table A or Table F, variants that include one or more folding mutations and fragments of these proteins that are fluorescent, such as Aequorea green fluorescent protein from which the two amino-terminal amino acids have been removed. Several of these contain different aromatic amino acids within the central chromophore and fluoresce at a distinctly shorter wavelength than wild type species. For example, engineered proteins P4 and P4-3 contain (in addition to other mutations) the substitution Y66H, whereas W2 and W7 contain (in addition to other mutations) Y66W. Other mutations both close to the chromophore region of the protein and remote from it in primary sequence may affect the spectral properties of GFP and are listed in the first part of the table below.

Detailed Description Text (70):
Additional mutations in Aequorea-related fluorescent proteins, referred to as "folding mutations," improve the ability of fluorescent proteins to fold at higher temperatures, and to be more fluorescent when expressed in mammalian cells, but have little or no effect on the peak wavelengths of excitation and emission. It should be noted that these may be combined with mutations that influence the spectral properties of GFP to produce proteins with altered spectral and folding properties. Folding mutations include: F64L, V68L, S72A, and also T44A, F99S, Y145F, N146I, M153T or A, V163A, I167T, S175G, S205T and N212K.

Detailed Description Text (81):
One embodiment of the invention includes a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at Q69, wherein the functional engineered fluorescent protein has a different fluorescent property than Aequorea green fluorescent protein. Preferably, the substitution at Q69 is selected from the group of K, R, E and G. The Q69 substitution can be combined with other mutations to improve the properties of the protein, such as a functional mutation at S65.

Detailed Description Text (82):
One embodiment of the invention includes a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at E222, but not including E222G, wherein the functional engineered fluorescent protein has a different fluorescent property than Aequorea green fluorescent protein. Preferably, the substitution at E222 is selected from the group of N and Q. The E222 substitution can be combined with other mutations to

improve the properties of the protein, such as a functional mutation at F64.

Detailed Description Text (83):
One embodiment of the invention includes a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least a substitution at Y145, wherein the functional engineered fluorescent protein has a different fluorescent property than Aequorea green fluorescent protein. Preferably, the substitution at Y145 is selected from the group of W, C, F, L, E, H, K and Q. The Y145 substitution can be combined with other mutations to improve the properties of the protein, such as a Y66.

Detailed Description Text (84):
The invention also includes computer related embodiments, including computational methods of using the crystal coordinates for designing new fluorescent protein mutations and devices for storing the crystal data, including coordinates. For instance the invention includes a device comprising a storage device and, stored in the device, at least 10 atomic coordinates selected from the atomic coordinates listed in FIGS. 5-1 to 5-46. More coordinates can be stored depending on the complexity of the calculations or the objective of using the coordinates (e.g. about 100, 1,000, or more coordinates). For example, the number of coordinates will be desirable for more detailed representations of fluorescent protein structure. Typically, the storage device is a computer readable device that stores code that it receives as input to atomic coordinates, although other storage means as known in the at art are contemplated. The computer readable device can be a floppy disk or a hard drive.

Detailed Description Text (99):
The results from the structural determinations of various mutations at His148 suggests that specific mutations at this position can result in overall structural adjustments in the beta barrel that can directly affect both solvent accessibility and the volume of the binding pocket. Substitution of His148 for example to smaller amino acids such Q, N, G, A, L, V and I would therefore be predicted to increase solvent access to the chromophore and therefore improve binding of larger anions. Likewise substitution of His 148 with larger amino acids such as F or W would be likely to reduce anion access to the chromophore. Similarly more subtle changes could be achieved by substituting positions 147 and 149 with smaller or larger amino acids.

Detailed Description Text (100):
These mutations will typically be introduced in the YFP template protein via oligo-mediated site directed mutagenesis to create libraries of mutant proteins that typically have a 10% probability of containing the wild-type amino acid residue and a 90% probability of containing one of the various mutant residues. Using this approach it is possible to rapidly screen libraries containing various combinations of mutants to identify the best combinations for a specific anion of interest. Typically this process can be repeated iteratively to ensure that sequence space around the binding pocket has been completely explored for any specific anion of interest.

Detailed Description Text (121):
DNA sequences encoding the fluorescence protein polypeptide of the invention can be expressed in vitro by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, in other words when the foreign DNA is continuously maintained in the host, are known in the art.

Detailed Description Text (123):
A primary advantage of fluorescent protein fusion proteins is that they are prepared by normal protein biosynthesis, thus completely avoiding organic synthesis and the requirement for customized unnatural amino acid analogs. The constructs can be expressed in E. coli in large scale for in vitro assays. Purification from bacteria is simplified when the sequences include polyhistidine tags for one-step purification by nickel-chelate chromatography. Alternatively, the substrates can be expressed directly in a desired host cell for assays in situ.

Detailed Description Text (136):
The engineered fluorescent proteins of this invention are useful in applications involving FRET. Such applications can detect events as a function of the movement of fluorescent donors and acceptor towards or away from each other. One or both of the donor/acceptor pair can be a fluorescent protein. A preferred donor and receptor pair for FRET based assays is a donor with a T203I mutation and an acceptor with the mutation T203X, wherein X is an aromatic amino acid-39, especially T203Y, T203W, or T203H. In a particularly useful pair the donor contains the following mutations: S72A, K79R, Y145F, M153A and T203I (with a excitation peak of 395 nm and an emission peak of 511 nm) and the acceptor contains the following mutations S65G, S72A, K79R, and T203Y. This particular pair provides a wide separation between the excitation and emission peaks of the donor and provides good overlap between the donor emission spectrum and the acceptor excitation spectrum. Other red-shifted mutants, such as those described herein, can also be used as the acceptor in such a pair.

Detailed Description Text (148):
YFP variants and revertants were prepared using the PCR-based QuikChange.TM. Site-Directed Mutagenesis Kit (Stratagene, La Jolla, Calif.), according to the manufacturer's directions and using the YFP clone 10c as a template (Ormo et al., 1996). Mutations were verified by sequencing the entire gene, and all GFP variants were expressed and purified as described (Ormo et al., 1996).

Detailed Description Text (154):
The two data sets were processed with Denzo v1.9 and scaled using ScalePack (Otwinowski & Minor, 1997). The spacegroup is P2.sub.1 2.sub.1 2.sub.1, with unit cell parameters a=51.2, b=62.8, and c=68.7 .ANG. for the iodide soak, and a=51.7, b=62.6, and c=66.2 .ANG. for the chloride soak. The crystals are nearly isomorphous to YFP-H148G (Wachter et al., 1998) and GFP S65T crystals (Ormo et al., 1996) previously described, and the YFP-H148G coordinate file 2yfp (Wachter et al., 1998) was used as a model for phasing. A model for the anionic chromophore was obtained by semi-empirical molecular orbital calculations using AM1 in the program SPARTAN version 4.1 (Wavefunction Inc., Irvine, Calif.).

Detailed Description Text (163):
As a step in understanding the properties of GFP, and to aid in the tailoring of GFPs with altered characteristics, we have determined the three dimensional structure at 1.9 .ANG. resolution of the S65T mutant (R. Heim et al. Nature 373:664-665 (1995)) of A. victoria GFP. This mutant also contains the ubiquitous Q80R substitution, which accidentally occurred in the early distribution of the GFP cDNA and is not known to have any effect on the protein properties (M. Chalfie et al. Science 263:802-805 (1994)).

Detailed Description Text (164):
Histidine-tagged S65T GFP (R. Heim et al. Nature 373:664-665 (1995)) was overexpressed in JM109/pRSET.sub.B in 4 l YT broth plus ampicillin at 37.degree. C., 450 rpm and 5 l/min air flow. The temperature was reduced to 25.degree. C. at A.sub.595 =0.3, followed by induction with 1 mM isopropylthiogalactoside for 5h. Cell paste was stored at -80.degree. C. overnight, then was resuspended in 50 mM HEPES pH 7.9, 0.3 M NaCl, 5 mM 2-mercaptoethanol, 0.1 mM phenylmethyl-

sulfonylfluoride (PMSF), passed once through a French press at 10,000 psi, then centrifuged at 20 K rpm for 45 min. The supernatant was applied to a Ni-NTA-agarose column (Qiagen), followed by a wash with 20 mM imidazole, then eluted with 100 mM imidazole. Green fractions were pooled and subjected to chymotryptic (Sigma) proteolysis (1:50 w/w) for 22 h at RT. After addition of 0.5 mM PMSF, the digest was reapplied to the Ni column. N-terminal sequencing verified the presence of the correct N-terminal methionine. After dialysis against 20 mM HEPES, pH 7.5 and concentration to A.sub.490 =20, rod-shaped crystals were obtained at RT in hanging drops containing 5 .mu.l protein and 5 .mu.l well solution, 22-26% PEG 4000 (Serva), 50 mM HEPES pH 8.0-8.5, 50 mM MgCl.sub.2 and 10 mM 2-mercapto-ethanol within 5 days. Crystals were 0.05 mm across and up to 1.0 mm long. The space group is P2.sub.1 2.sub.1 2.sub.1 with a=51.8, b=62.8, c=70.7 .ANG., Z=4. Two crystal forms of wild-type GFP, unrelated to the present form, have been described by M. A. Perrozo, K. B. Ward, R. B. Thompson, & W. W. Ward. J. Biol. Chem. 203, 7713-7716 (1988).

Detailed Description Text (165):
The structure of GFP was determined by multiple isomorphous replacement and anomalous scattering (Table E), solvent flattening, phase combination and crystallographic refinement. The most remarkable feature of the fold of GFP is an eleven stranded .beta.-barrel wrapped around a single central helix (FIGS. 1A and 1B), where each strand consists of approximately 9-13 residues. The barrel forms a nearly perfect cylinder 42 .ANG. long and 24 .ANG. in diameter. The N-terminal half of the polypeptide comprises three anti-parallel strands, the central helix, and then 3 more anti-parallel strands, the latter of which (residues 118-123) is parallel to the N-terminal strand (residues 11-23). The polypeptide backbone then crosses the "bottom" of the molecule to form the second half of the barrel in a five-strand Greek Key motif. The top end of the cylinder is capped by three short, distorted helical segments, while one short, very distorted helical segment caps the bottom of the cylinder. The main-chain hydrogen bonding lacing the surface of the cylinder very likely accounts for the unusual stability of the protein towards denaturation and proteolysis. There are no large segments of the polypeptide that could be excised while preserving the intactness of the shell around the chromophore. Thus it would seem difficult to re-engineer GFP to reduce its molecular weight (J. Dopf & T. M. Horiagon Gene 173:39-43 (1996)) by a large percentage.

Detailed Description Text (167):
The opposite side of the chromophore is packed against several aromatic and polar side chains. Of particular interest is the intricate network of polar interactions with the chromophore (FIG. 2C). His.sup.148, Thr.sup.203 and Ser.sup.205 form hydrogen bonds with the phenolic hydroxyl; Arg.sup.96 and Gln.sup.94 interact with the carbonyl of the imidazolidinone ring and Glu.sup.222 forms a hydrogen bond with the side chain of Thr.sup.65. Additional polar interactions, such as hydrogen bonds to Arg.sup.96 from the carbonyl of Thr.sup.62, and the side-chain carbonyl of Gln.sup.183, presumably stabilize the buried Arg.sup.96 in its protonated form. In turn, this buried charge suggests that a partial negative charge resides on the carbonyl oxygen of the imidazolidinone ring of the deprotonated fluorophore, as has previously been suggested (W. W. Ward. Bioluminescence and Chemiluminescence (M. A. DeLuca and W. D. McElroy, eds) Academic Press pp. 235-242 (1981); W. W. Ward & S. H. Bokman. Biochemistry 21:4535-4540 (1982); W. W. Ward et al. Photochem. Photobiol. 35:803-808 (1982)). Arg.sup.96 is likely to be essential for the formation of the fluorophore, and may help catalyze the initial ring closure. Finally, Tyr.sup.145 shows a typical stabilizing edge-face interaction with the benzyl ring. Trp.sup.57, the only tryptophan in GFP, is located 13 .ANG. to 15 .ANG. from the chromophore and the long axes of the two ring systems are nearly parallel. This indicates that efficient energy transfer to the latter should occur, and explains why no separate tryptophan emission is observable (D. C. Prasher et al. Gene 111:229-233 (1992). The two cysteines in GFP, Cys.sup.48 and Cys.sup.70, are 24 .ANG. apart, too distant to form a disulfide bridge. Cys.sup.70 is buried,

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but Cys.sup.48 should be relatively accessible to sulfhydryl-specific reagents. Such a reagent, 5,5'-dithiobis(2-nitrobenzoic acid), is reported to label GFP and quench its fluorescence (S. Inouye & F. I. Tsuji FEBS Lett. 351:211-214 (1994)). This effect was attributed to the necessity for a free sulfhydryl, but could also reflect specific quenching by the 5-thio-2-nitrobenzoate moiety that would be attached to Cys.sup.48.

Detailed Description Text (168):
Although the electron density map is for the most part consistent with the proposed structure of the chromophore (D. C. Prasher et al. Gene 111:229-233 (1992); C. W. Cody et al. Biochemistry 32:1212-1218 (1993)) in the cis [Z-] configuration, with no evidence for any substantial fraction of the opposite isomer around the chromophore double bond, difference features are found at >4 a in the final (F.sub.o -F.sub.c) electron density map that can be interpreted to represent either the intact, uncyclized polypeptide or a carbinolamine (inset to FIG. 2C). This suggests that a significant fraction, perhaps as much as 30% of the molecules in the crystal, have failed to undergo the final dehydration reaction. Confirmation of incomplete dehydration comes from electrospray mass spectrometry, which consistently shows that the average masses of both wild-type and S65T GFP (31,086.+-.4 and 31,099.5.+-.4 Da, respectively) are 6-7 Da higher than predicted (31,079 and 31,093 Da, respectively) for the fully matured proteins. Such a discrepancy could be explained by a 30-35% mole fraction of apoprotein or carbinolamine with 18 or 20 Da higher molecular weight The natural abundance of .sup.13 C and .sup.2 H and the finite resolution of the Hewlett-Packard 5989B electrospray mass spectrometer used to make these measurements do not permit the individual peaks to be resolved, but instead yields an average mass peak with a full width at half maximum of approximately 15 Da. The molecular weights shown include the His-tag, which has the sequence MRGSHHHHHH GMASMTGGQQM GRDLYDDDDK DPPAEF (SEQ ID NO:5). Mutants of GFP that increase the efficiency of fluorophore maturation might yield somewhat brighter preparations. In a model for the apoprotein, the Thr.sup.65 -Tyr.sup.66 peptide bond is approximately in the .alpha.-helical conformation, while the peptide of Tyr.sup.66 -Gly.sup.67 appears to be tipped almost perpendicular to the helix axis by its interaction with Arg.sup.96. This further supports the speculation that Arg.sup.96 is important in generating the conformation required for cyclization, and possibly also for promoting the attack of Gly.sup.67 on the carbonyl carbon of Thr.sup.65 (A. B. Cubitt et al. Trends Biochem. Sci. 20:448-455 (1995)).

Detailed Description Text (169):
The results of previous random mutagenesis have implicated several amino acid side chains to have substantial effects on the spectra and the atomic model confirms that these residues are close to the chromophore. The mutations T203I and E222G have profound but opposite consequences on the absorption spectrum (T. Ehrig et al. FEBS Letters 367:163-166 (1995)). T203I (with wild-type Ser.sup.65) lacks the 475 nm absorbance peak usually attributed to the anionic chromophore and shows only the 395 nm peak thought to reflect the neutral chromophore (R. Heim et al. Proc. Natl. Acad. Sci. USA 91:12501-12504 (1994); T. Ehrig et al. FEBS Letters 367:163-166 (1995)). Indeed, Thr.sup.203 is hydrogen-bonded to the phenolic oxygen of the chromophore, so replacement by Ile should hinder ionization of the phenolic oxygen. Mutation of Glu.sup.222 to Gly (T. Ehrig et al. FEBS Letters 367:163-166 (1995)) has much the same spectroscopic effect as replacing Ser.sup.65 by Gly, Ala, Cys, Val, or Thr, namely to suppress the 395 nm peak in favor of a peak at 470-490 nm (R. Heim et al. Nature 373:664-665 (1995); S. Delagrave et al. Bio/Technology 13:151-154 (1995)). Indeed Glu.sup.222 and the remnant of Thr.sup.65 are hydrogen-bonded to each other in the present structure, probably with the uncharged carboxyl of Glu.sup.222 acting as donor to the side chain oxygen of Thr.sup.65. Mutations E222G, S65G, S65A, and S65V would all suppress such H-bonding. To explain why only wild-type protein has both excitation peaks, Ser.sup.65, unlike Thr.sup.65, may adopt a conformation in which its hydroxyl donates a hydrogen bond to and stabilizes Glu.sup.222 as an anion, whose charge then inhibits ionization of the

chromophore. The structure also explains why some mutations seem neutral. For example, Gln.sup.80 is a surface residue far removed from the chromophore, which explains why its accidental and ubiquitous mutation to Arg seems to have no obvious intramolecular spectroscopic effect (M. Chalfie et al. Science 263:802-805 (1994)).

Detailed Description Text (170):
The development of GFP mutants with red-shifted excitation and emission maxima is an interesting challenge in protein engineering (A. B. Cubitt et al. Trends Biochem. Sci. 20:448-455 (1995); R. Heim et al. Nature 373:664-665 (1995); S. Delagrave et al. Bio/Technology 13:151-154 (1995)). Such mutants would also be valuable for avoidance of cellular autofluorescence at short wavelengths, for simultaneous multicolor reporting of the activity of two or more cellular processes, and for exploitation of fluorescence resonance energy transfer as a signal of protein-protein interaction (R. Heim & R. Y. Tsien. Current Biol. 6:178-182 (1996)). Extensive attempts using random mutagenesis have shifted the emission maximum by at most 6 nm to longer wavelengths, to 514 nm (R. Heim & R. Y. Tsien. Current Biol. 6:178-182 (1996)); previously described "red-shifted" mutants merely suppressed the 395 nm excitation peak in favor of the 475 nm peak without any significant reddening of the 505 nm emission (S. Delagrave et al. Bio/Technology 13:151-154 (1995)). Because Thr.sup.203 is revealed to be adjacent to the phenolic end of the chromophore, we mutated it to polar aromatic residues such as His, Tyr, and Trp in the hope that the additional polarizability of their systems would lower the energy of the excited state of the adjacent chromophore. All three substitutions did indeed shift the emission peak to greater than 520 nm (Table F). A particularly attractive mutation was T203Y/S65G/V68L/S72A, with excitation and emission peaks at 513 and 527 nm respectively. These wavelengths are sufficiently different from previous GFP mutants to be readily distinguishable by appropriate filter sets on a fluorescence microscope. The extinction coefficient, 36,500 M.sup.1-1 cm.sup.-1, and quantum yield, 0.63, are almost as high as those of S65T (R. Heim et al. Nature 373:664-665 (1995)).

Detailed Description Text (171):
Comparison of Aequorea GFP with other protein pigments is instructive. Unfortunately, its closest characterized homolog, the GFP from the sea pansy Renilla reniformis (O. Shimomura and F. H. Johnson J. Cell. Comp. Physiol. 59:223 (1962); J. G. Morin and J. W. Hastings, J. Cell. Physiol. 77:313 (1971); H. Morise et al. Biochemistry 13:2656 (1974); W. W. Ward Photochem. Photobiol. Reviews (Smith, K. C. ed.) 4:1 (1979); W. W. Ward. Bioluminescence and Chemiluminescence (M. A. DeLuca and W. D. McElroy, eds) Academic Press pp. 235-242 (1981); W. W. Ward & S. H. Bokman Biochemistry 21:4535-4540 (1982); W. W. Ward et al. Photochem. Photobiol. 35:803-808 (1982)), has not been sequenced or cloned, though its chromophore is derived from the same FSYG sequence as in wild-type Aequorea GFP (R. M. San Pietro et al. Photochem. Photobiol. 57:63S (1993)). The closest analog for which a three dimensional structure is available is the photoactive yellow protein (PYP, G. E. O. Borgstahl et al. Biochemistry 34:6278-6287 (1995)), a 14-kDa photoreceptor from halophilic bacteria. PYP in its native dark state absorbs maximally at 446 nm and transduces light with a quantum yield of 0.64, rather closely matching wild-type GFP's long wavelength absorbance maximum near 475 nm and fluorescence quantum yield of 0.72-0.85. The fundamental chromophore in both proteins is an anionic p-hydroxycinnamyl group, which is covalently attached to the protein via a thioester linkage in PYP and a heterocyclic iminolactam in GFP. Both proteins stabilize the negative charge on the chromophore with the help of buried cationic arginine and neutral glutamic acid groups, Arg.sup.52 and Glu.sup.46 in PYP and Arg.sup.96 and Glu.sup.222 in GFP, though in PYP the residues are close to the oxyphenyl ring whereas in GFP they are nearer the carbonyl end of the chromophore. However, PYP has an overall .alpha./.beta. fold with appropriate flexibility and signal transduction domains to enable it to mediate the cellular phototactic response, whereas GFP is a much more regular and rigid .beta.-barrel to minimize parasitic dissipation of the excited state energy as thermal or

conformational motions. GFP is an elegant example of how a visually appealing and extremely useful function, efficient fluorescence, can be spontaneously generated from a cohesive and economical protein structure.

Detailed Description Text (172):
A. Summary of GFP Structure Determination

Detailed Description Text (175):
The mutations F64L, V68L and S72A improve the folding of GFP at 37.degree. (B. P. Cormack et al. Gene 173:33 (1996)) but do not significantly shift the emission spectra.

Detailed Description Text (187):
The phenolic hydroxyl of Tyr203 has shifted towards the iodide-containing cavity by 0.6 .ANG., likely to improve the hydrogen bonding interaction with the halide (FIG. 12). There appears to be some flexibility in positioning the Tyr203 side chain next to the chromophore, presumably since it protrudes into a large water-filled cavity originally identified in the structure of GFP S65T (Ormo et al., 1996). An C.sub..alpha. -carbon overlay of 5 structures of YFP and its variants (Wachter et al., 1998) shows that the C.sub..beta. s of Tyr203 overlay quite well, whereas the phenolic oxygen varies by up to 1.4 .ANG.. The hydrogen bond between Tyr203 and the halide appears to be of major importance in the generation of a halide binding site with reasonably tight affinity (see mutational analysis below). The chromophore shift toward the halide may also serve to improve aromatic edge interactions with the anion. As a consequence, the carboxylate of Glu222 has rotated away from the chromophore ring nitrogen (distance increases from 3.3 to 3.6 .ANG.), and is now involved in a tight hydrogen bond to Ser205 (FIG. 12).

Detailed Description Text (189):
The buried iodide site in YFP-H148Q identifies a small cavity that is present in a number of structures examined and does not vary much in size (FIG. 13). Calculating van der Waals volumes using a sphere with a probe radius of 1.2 .ANG. (Connolly, 1985), the volume of this cavity is 21 .ANG..sup.3 in WT GFP (Brejc et al., 1997), 19 .ANG..sup.3 in GFP S65T (Ormo et al., 1996), 16 .ANG..sup.3 in YFP and YFP-H148G (Wachter et al., 1998), and 21 .ANG..sup.3 in YFP-H148G soaked in 500 mM KBr, where the crystallographic analysis shows that the binding site is also empty (unpublished data). The position of these cavities is essentially the same in the GFPs listed above, with its center close to Val150, Val163, Leu201, Ile152, Gln183, and Gln69, but about 6.6 .ANG. distant from the chromophore methylene bridge and 6.1 .ANG. from Arg96. WT (see below) and S65T GFP (Wachter & Remington, 1999) do not appear to interact with NaCl. On the other hand, all YFPs examined show anion interactions, with tightest Cl.sup.- binding observed for YFP (see below). Clearly, cavity size and position are not directly correlated with Cl.sup.- binding.

Detailed Description Text (206):
Revertants 3 (S72A/T203Y) and 4 (T203Y) were more difficult to analyze, since their titration behavior is similar to WT GFP. Their absorbance spectra exhibit a mixed ground state of bands A and B, and are nearly pH-independent above pH 6.5. Excitation of either band A or B leads to green fluorescence in these revertants, reminiscent of the excited-state deprotonation described for WT GFP (Chattoraj et al., 1996). Addition of NaCl to 250 mM to revertants 3 and 4 at pH 6.5 changes the ratio of the two absorbance bands only to a small degree, resulting in roughly a 20% decrease of band B in favor of band A. In WT GFP at pH 6.5, no spectral change is observed upon addition of 250 mM NaCl under conditions of constant ionic strength, consistent with a sensitivity towards ionic strength (Ward et al., 1982) but not specific anion binding.

Detailed Description Text (208):
Publications Baird, G. S., Zacharias, D. A. & Tsien, R. Y. (1999). Circular permutation and receptor insertion within green fluorescent proteins. Proc. Natl.

Acad. Sci. USA 96, 11241-11246. Bell, A. F., He, X., Wachter, R. M. & Tonge, P. J. (2000). Probing the ground state structure of the green fluorescent protein chromophore using Raman spectroscopy. Biochemistry (in press). Bokman, S. H. & Ward, W. W. (1981). Renaturation of Aequorea green fluorescent protein. Biochem. Biophys. Res. Commun. 101, 1372-1380. Born, M. (1920). Volumen und hydratationswaerme der ionen. Z. Physik 1, 45-48. Brejc, K., Sixma, T. K., Kitts, P. A., Kain, S. R., Tsien, R. Y., Ormo, M. & Remington, S. J. (1997). Structural basis for dual excitation and photoisomerization of the Aequorea victoria Green Fluorescent Protein. Proc. Natl. Acad. Sci. USA. 94, 2306-2311. Burley, S. K. & Petsko, G. A. (1988). Weakly polar interactions in proteins. Adv. Protein Chem. 39, 125-189. Cantor, C. R. & Schimmel, P. R. (1980). Biophysical Chemistry Part III. The behavior of biological macromolecules, W. H. Freeman and Company, New York, pp 866-878. Chattoraj, M., King, B. A., Bublitz, G. U. & Boxer, S. G. (1996). Ultra-fast excited state dynamics in Green Fluorescent Protein: Multiple states and proton transfer. Proc. Natl. Acad. Sci. USA 93, 8362-8367. Collaborative Computational Project N. 4 (1994). The CCP4 Suite: Programs for protein crystallography. Acta Cryst. D50, 760-763. Connolly, M. L. (1983). Solvent-accessible surfaces of proteins and nucleic acids. Science 221(4612), 709-713. Connolly, M. L. (1985). Computation of molecular volume. J. Am. Chem. Soc. 107, 1118-1124. De Giorgi, F., Ahmed, Z., Bastianutto, C., Brini, M., Jouvaille, L. S., Marsault, L. S., Murgia, M., Pinton, P., Pozzan, T. & Rizzutto, R. (1999). Meth. Cell Biol. 58, 75-85. Doi, N. & Yanagawa, H. (1999). Design of generic biosensors based on green fluorescent proteins with allosteric sites by directed evolution. FEBS Lett. 453, 305-307. Elsliger, M.-A., Wachter, R. M., Hanson, G. T., Kallio, K. & Remington, S. J. (1999). Structural and spectral response of green fluorescent protein variants to changes in pH. Biochemistry 38, 5296-5301. Feller, G., le Bussy, O., Houssier, C. & Gerday, C. (1996). Structural and functional aspects of chloride binging to Alteromonas haloplanctis alpha-amylase. J. Biol. Chem. 271, 23836-23841. Fersht, A. (1985). Enzyme structure and mechanism, W. H. Freeman and Company, New York, N.Y., pg 298. Halm, D. R. & Frizzell, R. A. (1992). Anion permeation in an apical membrane chloride channel of a secretory epithelial cell. J. Gen. Physiol. 99, 339-366. Heim, R. (1999). Green fluorescent protein forms for energy transfer. Methods Enzymol. 302, 408-423. Heim, R., Prasher, D. C. & Tsien, R. Y. (1994). Wavelength mutations and posttranslational autoxidation of green fluorescent protein. Proc. Natl. Acad. Sci. USA 91, 12501-12504. Jayaraman, S., Haggie, P., Wachter, R. M., Remington, S. J. & Verkman, A. S. (2000). Mechanism and cellular applications of a green fluorescent protein-based halide sensor. J. Biol. Chem. (in press). Jones, T. A., Zou, J.-Y., Cowan, S. W. & Kjelgaard, M. (1991). Improved methods for building protein models in electron density maps and the location of errors in these models. Acta Crystallogr. Sect. A 47, 110-119. Kneen, M., Farinas, J., Li, Y. & Verkman, A. S. (1998). Green fluorescent protein as a noninvasive intracellular pH indicator. Biophys. J. 74, 1591-1599. Kraulis, P. (1991). MOLSCRIPT: A program to produce both detailed and schematic plots of protein structures. J. Appl. Crystallog. 24, 946-950. Llopis, J., McCaffery, J. M., Miyawaki, A., Farquhar, M. & Tsien, R. Y. (1998). Measurement of cytosolic, mitochondrial, and Golgi pH in single living cells with green fluorescent proteins. Proc. Natl. Acad. Sci. USA 95, 6803-6808. March, J. (1992). Advanced Organic Chemistry, John Wiley & Sons, New York, pg 272. Matz, M. V., Fradkov, A. F., Labas, Y. A., Savitsky, A. P., Zaraisky, A. G., Markelov, M. L. & Lukyanov, S. A. (1999). Fluorescent proteins from nonbioluminescent Anthozoa species. Nature Biotechnol. 17, 969-973. Miesenbock, G., De Angelis, D. A. & Rothman, J. E. (1998). Visualizing secretion and synaptic transmission with pH-sensitive green fluorescent proteins. Nature 394, 192-195. Miyawaki, A., Griesbeck, O., Heim, R. & Tsien, R. Y. (1999). Dynamic and quantitative Ca2+ measurements using improved cameleons. Proc. Natl. Acad. Sci. USA 96, 2135-2140. Ormo, M., Cubitt, A. B., Kallio, K., Gross, L. A., Tsien, R. Y. & Remington, S. J. (1996). Crystal structure of the Aequorea victoria Green Fluorescent Protein. Science 273, 1392-1395. Otwinowski, Z. & Minor, W. (1997). Processing of X-ray diffraction data collected in oscillation mode. Methods Enzymol. 276, 307-326. Palm, G. J. & Wlodawer, A. (1999). Spectral variants of green fluorescent protein. Methods Enzymol. 302, 378-394. Palm, G. J., Zdanov, A.,

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Detailed Description Paragraph Table (2):
TABLE A Excitation Emission Extinct. Coeff. (M.sup.-1 Quantum Clone Mutation(s) max (nm) max (nm) cm.sup.-1) yield Wild type None 395 (475) 508 21,000 (7,150) 0.77 P4 Y66H 383 447 13,500 0.21 P4-3 Y66H 381 445 14,000 0.38 Y145F P4-3E Y66H 384 448 22,000 0.27 Y145F V163A W7 Y66W 433 (453) 475 (501) 18,000 (17,100) 0.67 N146I M153T V163A N212K W2 Y66W 432 (453) 480 10,000 (9,600) 0.72 I123V Y145H H148R M153T V163A N212K W1C S65A 435 495 21,200 0.39 Y66W S72A N146I M153T V163A W1B F64L 434 (452) 476 (505) 32,500 0.4 S65T Y66W N146I M153T V163A S65T S65T 489 511 39,200 0.68 P4-1 S65T 504 (396) 514 14,500 (8,600) 0.53 M153A K238E Emerald S65T, S72A, 487 509 57,500 0.68 N149K, M153T, I167T EGFP F64L, S65T 488 507 55,900 0.64 S65A S65A 471 504 S65C S65C 479 507 S65L S65L 484 510 Y66F Y66F 360 442 Y66W Y66W 458 480 Topaz S65G 514 527 94,500 0.6 S72A K79R T203Y 10C S65G 514 527 83,400 0.61 YFP V68L S72A T203Y Sapphire S72A, Y145F 399 511 29,000 0.64 T203I

Detailed Description Paragraph Table (5):
TABLE D Original position and presumed role Change to Codon L220 Packs next to Glu222; to make GFP pH sensitive HKNPQT MMS KKS V224 Packs next to Glu222; to make GFP pH sensitive HKNPQT MMS KKS CFHLQRWYZ YDS RHS

Detailed Description Paragraph Table (7):
TABLE F Original position and presumed role Change to i) Mutation of amino acids around the ion binding pocket to increase binding affinity for smaller anions than iodide. V150 Lines binding pocket S, T, Q, N I152 Lines binding pocket L, V, F, S, T, Q, N V163 Lines binding pocket S, T, Q, N F165 Lines binding pocket Y, W H181 Lines binding pocket F, W Q183 Lines binding pocket K, R, N L201 Lines binding pocket S, T, Q, N, V, I ii) Mutation of amino acids around the ion binding pocket to increase binding affinity for larger anions than iodide. V150 Lines binding pocket A, C, M, G, S, L I152 Lines binding pocket A, C, M, G, S V163 Lines binding pocket A, C, M, G, S, L F165 Lines binding pocket Y, L H181 Lines binding pocket K, R Q183 Lines binding pocket N, S, C L201 Lines binding pocket A, C, M, G, S

Detailed Description Paragraph Table (10):
TABLE H Extinction Excitation coefficient Emission Clone Mutations max. (nm) (10.sup.3 M.sup.-1 cm.sup.-1) max. (nm) S65T S65T 489 39.2 511 5B T203H/S65T 512 19.4 524 6C T203Y/S65T 513 14.5 525 10B T203Y/F64L/S65G/S72A 513 30.8 525 10C T203Y/F65G/V68L/S72A 513 36.5 527 11 T203W/S65G/S72A 502 33.0 512 12H T203Y/S65G/S72A 513 36.5 527 20A T203Y/S65G/V68L/Q69K/S72A 515 46.0 527